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STUDIES IN MILK RECORDS: ON THE ACCURACY OF ESTIMATING A COW'S MILKING CAPABILITY BY HER FIRST LACTATION YIELD.

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A GOOD deal of uncertainty seems to exist among dairy farmers as to how far the first lactation yield may be taken as a guide to a cow's future milking career.

All know of course that it is by no means an infallible one, while some go so far as to ignore it altogether, and postpone "weeding out" bad cows until after the second calf is born. In any case it is obviously a matter of some practical importance that the limits of its accuracy should, so far as is possible, be ascertained.

The present work is based on 336 cows which have had five or more calves (about 2240 lactation records in all). Every cow is included in every calculation unless suffering from abortion or serious illness.

The "revised maximum" has been used throughout as the measure expressing a cow's yield for any given lactation. It may be defined as the maximum day-yield of the lactation which is three times reached (or exceeded)[1]. The advantages of using this figure, and its relationship to more ordinary methods of measurement as by lactation totals, have been discussed in a previous paper[2].

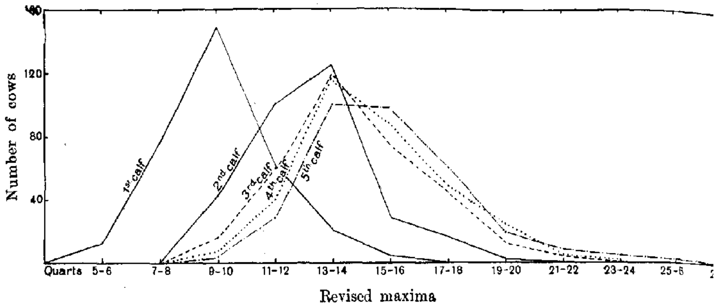
The cows represented are principally British Holsteins and non-pedigree dairy Shorthorns.

The revised maxima given with successive calves were first considered separately, *i.e.* all the first calf R. M. together, all the second calf R. M.,

[1] In other words, the highest figure common to the three highest day-yields of a lactation.

[2] Gavin, "The Interpretation of Milk Records," *Journ. Roy. Agric. Soc.* 1912, p. 153; *v.* also "Studies in Milk Records: Influence of Foetal Growth on Yield," *Journ. Agric. Science*, 1913, Vol. v. Part 3.

and so on. The frequency curves obtained for 1st to 5th calf are given in the following diagram.



Frequency curves. Revised maxima. 320 cows.

With *first* calf — —

" *second* " ·· ·· —·——···· ··

" *third* " - - - - - - - - - - -

" *fourth* " . . . .

" *fifth* " —·—·—·—·

Constants of variation of R.M. *given with successive calves.*

Lactation	No. of records	Mean	Median	Mode	Modal coefficient	Standard deviation	Coefficient of variation
		quarts	quarts	quarts	per cent.	quarts	
First	320	9·3 ± 0·074	9·50 ± 0·092	9·9	47	1·97 ± 0·052	21·2 ± 0·56
Second	313	12·8 ± 0·081	12·77 ± 0·101	12·71	39	2·12 ± 0·057	16·6 ± 0·45
Third	326	14·2 ± 0·094	13·97 ± 0·117	13·51	36	2·53 ± 0·067	17·8 ± 0·47
Fourth	328	14·9 ± 0·091	14·54 ± 0·114	13·82	35	2·46 ± 0·065	16·6 ± 0·44
Fifth	323	15·4 ± 0·104	15·08 ± 0·130	14·44	31	2·73 ± 0·072	17·8 ± 0·47

A class unit of two quarts was taken in all cases.

The mode was calculated from the formula

$$\text{Mode} = \text{mean} - 3\,(\text{mean} - \text{median}),$$

which gives a reasonably correct value in most cases[1]. The modal coefficient is the percentage of variates falling on the empirical mode, and the coefficient of variation[2] $= \dfrac{100\sigma}{\text{mean}}$.

[1] Pearson, *Biometrika*, Vol. I. 1902, p. 260.

[2] Pearson, 'Regression Heredity and Panmixia," *Phil. Trans. Roy. Soc.* CLXXXVII. 1896, p. 276.

The usual formulae, which of course can only be said to be strictly correct in cases of normal distribution, were used for calculation of probable errors.

The small variation in the number of cows which could be included in the different lactation calculations (due to abortion or to serious illness at the time when it seemed probable they would have given their maximum yield) has been eliminated in the diagram on p. 378, where all the curves have been reduced to the ratio of 320 cows.

From the table of constants it is seen that the mean continues to increase right up to the fifth calf. Beyond the fifth calf the following figures were obtained:

Lactation	Mean R.M.	No. of cows
Sixth	15·85	221
Seventh	15·51	148
Eighth	15·48	83

but these cannot be strictly comparable with the others, since as soon as one ceases to deal with the same number of cows throughout, the influence of selection must come in. It is probable that only the best or the healthiest of the 336 cows would tend to remain in the herd after their fifth calf.

The coefficient of variation is 21·2 with first calf, after which it falls to the neighbourhood of 17. Determinations made from a smaller number of cows revealed a tendency to rise again after the fifth calf.

The increased variability of first lactation yield seems to the writer more likely to be due to the greatly varying condition in which heifers are brought into the milking shed than to have any physiological basis. Differences in feeding and general treatment and, most of all, in age, must influence very greatly the first lactation yield, and this influence must tend to decrease as the milking career advances.

With uniform treatment and uniform age it seems probable therefore that this extra variability could at any rate be reduced, but for the general conditions of farm practice it must be recognised as a factor affecting the accuracy of estimates based on first lactation yields.

In considering the correlation between the first and subsequent lactation yields, it becomes necessary to decide on a figure to represent a cow's mature capability. The writer has previously proposed taking the average 4th, 5th, and 6th calf R. M., but he now suggests substituting

for this the *maximum* R. M., that is to say, the largest daily yield ever given in the life of a cow, provided it is reached three times in the same lactation (this stipulation being to avoid abnormally high entries due to late milking, clerical errors, etc.).

The whole idea of taking maximum instead of average yields will perhaps be viewed with disfavour by some practical breeders as being less in accordance with actual results obtained. The importance, however, of aiming primarily at physiological capability must be borne in mind, and it is suggested that, in the same way as the maximum day-yield of any one lactation is the least variable function of the yield of that lactation[1] and probably the most reliable guide to the cow's capacity at the time, so the highest of these maxima to which she attains during her life will be the best and most comparable indication of her physiological capabilities in reasonably favourable circumstances.

The correlation coefficients between this maximum R. M. and the R. M. given with first five lactations are as follows:

Lactation	r with max. R. M.	Probable error
First	+ ·394	± 0·031
Second	+ ·452	± 0·030
Third	+ ·506	± 0·028
Fourth	+ ·605	± 0·024
Fifth	+ ·762	± 0·016

The average[2] constants of variation for the maximum R. M. are:

Mean	Median	Mode	Modal coefficient	Standard deviation	Coefficient of variation
17·14 qts.	16·70 qts.	15·82 qts.	32 %	2·88	16·8

It will be noticed that the second lactation figure is only ·058 higher than that given by first lactation, but this increase does not fully represent the accuracy that can be gained by keeping a cow until after

[1] *v.* Interpretation of Milk Records.

[2] Owing to the fact that all cows were not available for every correlation table, these constants shew slight variations. The actual values obtained were as follows:

Mean......	17·0	17·1	17·2	17·2	17·2
σ	2·85	2·75	2·98	2·95	2·94
v...........	16·8	16·1	17·3	17·2	17·1

Correlation table. First calf R.M. *and max.* R.M.

1st R.M. Quarts	Max. R.M. Quarts 12—13	14—15	16—17	18—19	20—21	22—23	24—25	26—27	28—29	Totals
5—6	—	8	3	—	—	—	—	—	—	11
7—8	5	30	26	13	1	—	—	—	—	75
9—10	6	42	52	34	8	3	1	1	2	149
11—12	1	5	16	14	16	2	—	3	2	59
13—14	—	5	8	3	2	1	2	—	—	21
15—16	—	—	—	2	2	—	—	—	—	4
17—18	—	—	—	—	—	1	—	—	—	1
Totals	12	90	105	66	29	7	3	4	4	320

Correlation table. Second calf R.M. *and max.* R.M.

2nd R.M. Quarts	Max. R.M. Quarts 12—13	14—15	16—17	18—19	20—21	22—23	24—25	26—27	28—29	Totals
9—10	4	16	12	5	5	—	—	—	—	42
11—12	4	38	40	15	3	1	—	—	—	101
13—14	2	29	39	30	17	2	2	—	2	123
15—16	—	1	12	10	4	—	—	1	—	28
17—18	—	—	1	4	6	2	—	2	1	16
19—20	—	—	—	—	—	1	1	—	—	2
21—22	—	—	—	—	—	1	—	—	—	1
Totals	10	84	104	64	35	7	3	3	3	313

Correlation table. Third calf R.M. *and max.* R.M.

3rd R.M. Quarts	Max. R.M. Quarts 12—13	14—15	16—17	18—19	20—21	22—23	24—25	26—27	28—29	Totals
9—10	4	6	5	1	—	—	—	—	—	16
11—12	5	30	11	8	4	—	1	1	—	60
13—14	5	39	43	22	7	—	1	—	1	118
15—16	—	12	32	17	7	6	—	—	—	74
17—18	—	1	11	16	9	1	1	1	3	43
19—20	—	—	—	2	6	1	—	1	1	11
21—22	—	—	—	—	2	1	—	—	—	3
23—24	—	—	—	—	—	—	—	—	—	—
25—26	—	—	—	—	—	—	—	—	—	—
27—28	—	—	—	—	—	—	—	1	—	1
Totals	14	88	102	66	35	9	3	4	5	326

Correlation table. Fourth calf R.M. *and max.* R.M.

4th R.M. Quarts	Max. R.M. Quarts 12—13	14—15	16—17	18—19	20—21	22—23	24—25	26—27	28—29	Totals
9—10	2	4	—	1	—	—	—	—	—	7
11—12	7	20	10	3	—	—	—	—	—	40
13—14	3	53	37	18	3	—	—	1	—	115
15—16	—	12	43	15	12	3	2	—	1	88
17—18	—	—	14	22	6	3	1	—	3	49
19—20	—	—	—	7	13	1	—	2	1	24
21—22	—	—	—	—	3	1	—	1	—	5
Totals	12	89	104	66	37	8	3	4	5	328

Correlation table. Fifth calf R.M. *and max.* R.M.

5th R.M. Quarts	Max. R.M. Quarts 12—13	14—15	16—17	18—19	20—21	22—23	24—25	26—27	28—29	Totals
9—10	2	3	—	—	—	—	—	—	—	5
11—12	4	16	6	2	—	—	—	—	—	28
13—14	5	49	35	9	1	1	—	—	—	100
15—16	—	20	48	20	10	—	—	—	—	98
17—18	—	—	11	31	15	2	—	—	—	59
19—20	—	—	—	5	8	—	1	1	3	18
21—22	—	—	—	—	4	2	1	1	1	9
23—24	—	—	—	—	—	2	1	—	—	3
25—26	—	—	—	—	—	—	—	2	—	2
27—28	—	—	—	—	—	—	—	—	1	1
Totals	11	88	100	67	38	7	3	4	5	323

her second calf. By taking the average[1] between 1st and 2nd R.M., the correlation coefficient with max. R.M. is raised to

$$+\cdot 526 \pm 0\cdot 028.$$

[1] Constants of variation given by this average are: Mean 11·18 quarts ± 0·071, σ 1·82 quarts ± 0·050, v 16·3 ± 0·448.

It should perhaps be emphasised that, in calculating this correlation, all available cows were used, as in the other cases. The cows used for determining the correlation of max. R.M. with R.M. of first lactation, of second lactation, and mean of first and second lactations are therefore not all the same (cf. variation in the constants for max. R.M. in the

It would appear that in some cases an abnormally low or high yield (abnormal, that is, for the cow in question—out of proportion, by reason of exterior circumstances, to its inherent capacity) is compensated for by an increase or reduction in the following lactation. Large fluctuations from year to year, even after a cow has reached maturity, are also very common indeed. Many of these would doubtless be explicable at the time by those having full knowledge of the attendant circumstances, but all must reduce the value of the correlation coefficients.

That these tend to be low, even between successive lactations, is shewn by the following results:

Correlation between	r	Probable error
First and second lactations	+·437	±0·031
Second and third ,,	·388	0·033
Third and fourth ,,	·576	0·025
Fourth and fifth ,,	·527	0·028

Thus compared with other relationships, that existing between 1st R. M. and max. R. M. assumes greater importance than the coefficient of ·394 would by itself indicate.

With regard to the estimation of mature from first calf yield, a simple factor will not suffice, since cows starting badly tend to increase to a greater proportion of their first calf yield than those which begin with a higher figure. Recourse must therefore be had to regression coefficients[1], which are given in the following table, together with the probable error of estimate[2].

footnote on p. 380). As a consequence if we calculate the s.d. of mean R.M. of first and second lactations from the formula $\frac{1}{2}(\sigma_1^2+\sigma_2^2+2r_{12}\sigma_1\sigma_2)^{\frac{1}{2}}$, we get 1·73, not 1·82: and if we calculate the correlation from the formula

$$\frac{r_{13}\sigma_1+r_{23}\sigma_2}{\sqrt{\sigma_1^2+\sigma_2^2+2r_{12}\sigma_1\sigma_2}},$$

we get 0·500, not 0·526. By reason of the coefficients not being entirely comparable with each other, the application of the theory of partial correlation to determine a regression equation giving the deviation in max. R.M. in terms of the deviations of first and second R.M. separately is a little doubtful. Mr Yule finds the equation

$$x_m = 0·345\,x_1 + 0·457\,x_2,$$

where x_m is the deviation in max. R.M. The probable error of estimate in using this equation is only very slightly lower than the p.e. of estimate in using the mean of first and second lactations.

[1] Regression coefficient of x relative to $y = r\frac{\sigma_x}{\sigma_y}$. Probable error of regression coefficient $= \frac{·6745\,\sigma_x}{\sigma_y}\sqrt{\frac{1-r^2}{n}}$.

[2] Probable error of estimate $= ·6745 \times \sigma\sqrt{1-r^2}$.

Regression of max. R.M. *relative to* R.M. 1—5.

Lactation	Regression coefficient	Probable error of estimate
		quarts
First	·57 ± 0·050	1·77
Second	·58 ± 0·046	1·66
Third	·60 ± 0·037	1·73
Fourth	·73 ± 0·035	1·58
Fifth	·82 ± 0·026	1·28
Average first and second	·79 ± 0·053	1·58

One example may be given of the use of the regression coefficient. A cow gives R.M. 7·3 quarts with first calf. What will be her max. R.M.? The mean of first calf R.M. is 9·3, *i.e.* she differs from that mean by -2. She will therefore differ from the mean of max. R.M., which is 17·14 quarts, by $-2 \times$ regression coefficient, that is, her average, or expected, max. R.M. will be

$$17{\cdot}14 - (2 \times {\cdot}57) = 17{\cdot}14 - 1{\cdot}14 = 16 \text{ quarts.}$$

Further, the chances are *even* that this estimate is correct within the limits of $\pm$ 1·8 quarts.

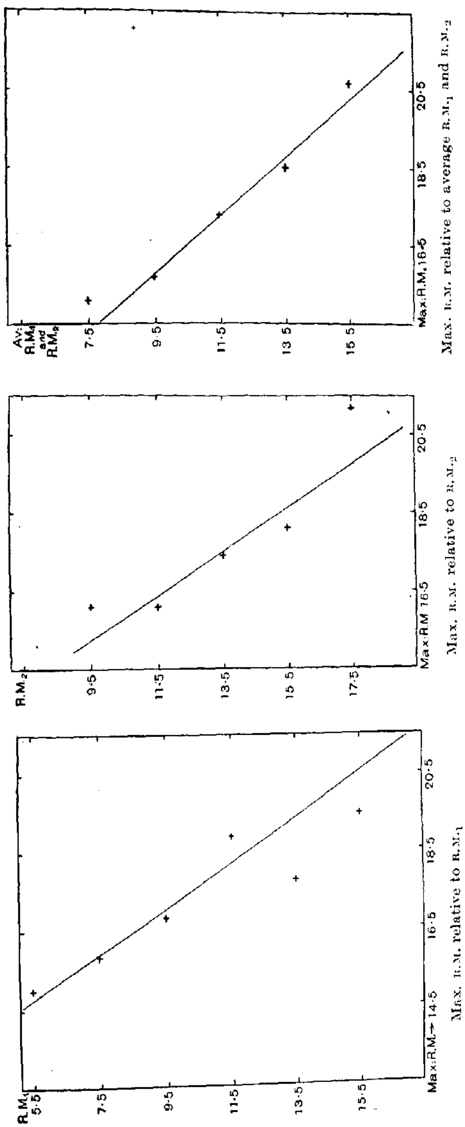
The accuracy of these calculations depends on the distribution of each array being normal, and on the means of arrays falling on the line representing characteristic regression. Unfortunately neither of these conditions are perfectly fulfilled, and the results cannot be regarded as more than the greatest approximation to accuracy so far obtained.

The following diagrams shew the line of characteristic regression, together with means of arrays for max. R.M. relative to (1) first calf R.M., (2) second calf R.M., and (3) average between first and second calf R.M. It will be seen that with (3) the regression is very nearly linear.

The results of the application of the regression coefficients are given in tabular form. A column has been added shewing the calculated best totals for a normal lactation. These have been derived from constants previously obtained in calculating the correlation between totals and R.M. of 1233 cows[1]. These were as follows:

1233 normal lactations		
Mean. Totals	656·4 gals. ± 3·24	
,, R.M.	13·87 qts. ± 0·07	
Correlation coefficient	+ ·844	± 0·005
Regression coefficient. Totals relative to R.M.	41·54	± 0·518
Probable error of estimate	± 60 gals.	

[1] *v.* Interpretation of Milk Records.



Lines of characteristic regression, with means of arrays.

Thus, in the following table, column 4 represents the *best* yield likely to be obtained from a cow giving the corresponding first calf R.M. (column 1) in a normal lactation of about 9 months, at an average season of calving. Cows calving from September to April would be inclined to yield about 50 gallons more, and those calving from May to August up to 50 gallons less[1].

Table for estimating max. R.M. from (a) 1st R.M. and (b) average 1st and 2nd R.M.

1st calf R.M.	Calculated max. R.M.	Limits of probable error	Lactation total[2] corresponding to max. R.M.
quarts	quarts	quarts	gallons
5	14·6	12·9—16·3	685
6	15·1	13·4—16·8	709
7	15·7	14·0—17·4	733
8	16·3	14·6—18·0	757
9	16·8	15·1—18·5	781
10	17·4	15·7—19·1	804
11	18·0	16·3—19·7	827
12	18·5	16·8—20·2	851
13	19·1	17·4—20·8	874
14	19·7	18·0—21·4	898
15	20·3	18·5—21·9	921
16	20·8	19·1—22·5	944
Average R.M. 1st and 2nd calves			
7	13·7	12·1—15·3	651
8	14·5	12·9—16·1	684
9	15·3	13·7—16·9	717
10	16·1	14·5—17·7	750
11	16·9	15·3—18·5	783
12	17·7	16·1—19·3	816
13	18·5	16·9—20·1	849
14	19·3	17·7—20·9	882
15	20·1	18·5—21·7	915
16	20·9	19·3—22·5	948

The next table has been compiled from an analysis of the correlation between max. R.M. and R.M._1. The former have been converted into the corresponding lactation totals, and the percentage of cows calculated which gave max. R.M. corresponding to less than 700, 700—800 and more than 800 gallons respectively. It must be emphasised that these

[1] *v.* Interpretation of Milk Records.

[2] The probable error of estimated lactation total is about ± 61 gallons, and is expressed by the formula $\sqrt{(\text{p.e.})_1^2 + (\text{r.c.})^2 (\text{p.e.})_2^2}$, where $(\text{p.e.})_1$ is P.E. of estimate of max. R.M., $(\text{p.e.})_2$ the P.E. of estimate of lactation total from any R.M., and r.c. the regression of totals relative to R.M. The author is indebted to Mr G. Udny Yule for information on this point.

are the *largest* yields the cows are ever likely to give in a normal lactation; their average yields would of course be considerably less. Such estimates must necessarily be approximate, but they may serve as a guide to the general proportions to be expected.

1st calf R.M.	Cows giving maximum total yield of		
	Less than 700 gals.	700—800 gals.	More than 800 gals.
	%	%	%
5—7 quarts	33	50	17
8—9 ,, ...	22	54	24
10—11 ,, ...	14	45	41
12—17 ,, ...	8	29	63
Less than 10 quarts	25	53	22
10 quarts...............	16	48	36
More than 10 quarts	8	33	59

One other method of examination has been employed. The cows were divided into three classes according to their first calf R.M. The mean R.M. with subsequent calves was then calculated for each class. The results are given in the following table and diagram (page 388).

1st calf R.M.	No. of cows	Mean R.M.					Average 1st—5th	
		1st	2nd	3rd	4th	5th	R.M.	Corresponding normal lactation total
		qts.	qts.	qts.	qts.	qts.		gallons
Class C. 5—9 qts.	153	8·1	11·3	13·7	14·4	15·4	12·6	604
,, B. 10—11 ,,	112	10·4	13·3	14·7	15·3	15·9	13·9	658
,, A. 12—17 ,,	50	13·0	14·9	16·1	16·4	16·9	15·5	724

These figures lead to some very significant conclusions. The average difference between classes C and B is 54 gallons, or a total of 270 gallons in the five years. At 8*d.* a gallon this comes to £9. Similarly calculated, the difference between classes A and B is £11. Thus, according to the present data an *average* cow of class A should give a return of £20 in five years over and above that given by an *average* cow of class C.

These three divisions of first calf R.M., namely (*c*) less than 10 quarts, (*b*) 10 and 11 quarts, and (*a*) 12 quarts and upwards, should be useful ones for the practical breeder to bear in mind. It certainly appears that cows of class C should be discarded, as far as possible, after their first calf, bearing in mind however the increased accuracy of a judgement

based on the mean of first and second calf R.M. Thus if any extenuating circumstances are present, a reprieve should be granted until after the second calf. The probability is that about one in five will turn out good yielders.

Class B offers the great opportunity to the skilful judge of dairy-cows. It is "odds on" that they will pay for keeping, but "odds against" that they will turn out high-yielders. Speaking generally, these cows should be kept for their second calf.

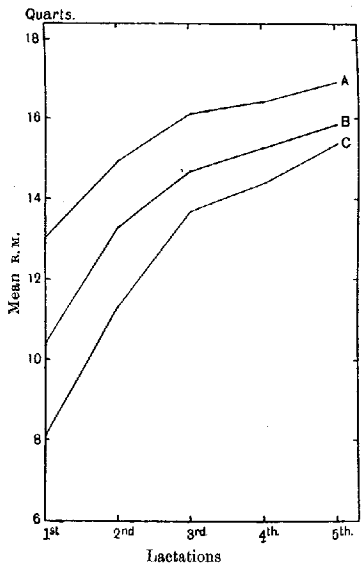


Diagram shewing subsequent average yield of cows classed as follows:

Class A.	Cows giving	1st R.M. of	17—12	quarts.
„ B.	„	„	11—10	„
„ C.	„	„	9—5	„

Cows of class A no dairy farmer will be likely to part with, and in spite of individual exceptions it is clear from the preceding diagram that cows starting well tend on the whole to maintain a good proportion, though not all, of their initial lead over the bad starters. At the same time it unfortunately appears to be less certain that class A will do well than that class C will do badly, as shewn by the following coefficients of variation:

Variability of max. R.M. given by cows belonging to		
Class C	Class B	Class A
13·5 ± 0·51	17·2 ± 0·78	16·9 ± 1·14

Classed according to 1st calf R.M.

There is another field for the practical application of such results. In the study of the inheritance of milk yield it becomes necessary to define a cow's mature capability by a single unqualified figure, and it has already been suggested that the max. R.M. should be chosen for this purpose.

In breeding, however, this implies many years delay, and in the examination of records the number available becomes very greatly reduced if all cows that did not reach maturity have to be excluded. It is advisable therefore that a uniform system should be formed to deal with the immature cows by calculating the max. R.M. from the data available in each case.

It is probable that calculation will give in many cases a less accurate result than a prognostication by a practical breeder, but in such work it seems essential that personal bias should be excluded.

SUMMARY.

(Lactations are measured throughout by R.M. in quarts.)

1. The first lactation shews greater variability than the second, third, fourth or fifth.
2. The estimation of one lactation from another cannot be made with great accuracy, since the correlation coefficient between even successive lactations does not rise above + ·6.
3. It is necessary, in classing a cow, to decide on one lactation that shall represent her mature capability. It is suggested that the maximum lactation is the most suitable one to chose.
4. The correlation coefficient with the maximum lactation increases from ·394 for first to ·762 for fifth lactation.
5. The mean of the first and second lactations however gives a correlation coefficient of ·526 with maximum lactation, which is higher than any of the first three taken separately.

6. The probable error of estimating maximum from first, second or third lactations is about 1·7 quarts. The inaccuracy is likely to be greater than this, since regression of the former has not yet been shewn to be strictly linear.

7. The mean of first and second lactations gives a probable error of estimate of 1·6 quarts and the regression of maximum lactation relative to this figure is very nearly linear.

8. Tables are given for estimating maximum lactation from both first lactation, and mean of first and second.

9. The general conclusion arrived at is that with cows giving a fairly high or fairly low first lactation R.M., this figure should be used to determine whether they shall be kept or not; but with cows giving a medium first lactation R.M. of 10—11 quarts, it is worth waiting to obtain the increased accuracy of an estimate based on the mean of first and second lactation R.M.

These investigations are being undertaken on behalf of Lord Rayleigh and the Hon E. G. Strutt with data accumulated by them during the last twenty years. For any deficiencies in method or treatment of the material, however, the author is alone responsible.

THE DISTRIBUTION OF ATMOSPHERIC IMPURITIES IN THE NEIGHBOURHOOD OF AN INDUSTRIAL CITY.

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IN a previous paper[1] a summary was given of the results obtained in an examination of the degree of pollution of the atmosphere in various parts of the city of Leeds, as diagnosed by the analysis of samples of rain collected at various centres. These results showed that, even in the cleaner suburbs of the city, the purity of the air compares very unfavourably with the standard of country districts remote from industrial areas.

An examination in similar fashion of the atmosphere in the country surrounding the city to a distance, in some directions, of seven miles from its centre, has now been completed, and the results are summarised and discussed in the following pages.

Throughout the twelve months from July 1, 1911, to July 1, 1912, samples of rain were collected at fourteen stations situated respectively with reference to the centre of Leeds as indicated in the following schedule (Table I) and diagram (Fig. 1).

Briefly summarised, the stations were so arranged as to give a complete ring of stations on a circle of three miles radius from the centre of the city, whilst to the north, north-east and east additional stations were selected at distances of five miles and seven miles respectively from the centre. Leeds is bordered in all directions, except the three above-mentioned, by a thickly populated industrial area, coal-mining and iron-working prevailing to the south-east and south, and woollen manufacturing to the south-west and west, whilst

[1] Crowther and Ruston, This *Journal*, IV. pp. 25—55.

large engineering works are situated throughout both areas. It was felt therefore that little was to be gained by collecting samples more than three miles out in these directions, since the existence of heavy and varied local pollution would almost hopelessly complicate the interpretation of the results. Thus, a station seven miles due west of Leeds would be well within the boundaries of the almost equally large industrial city of Bradford; at the same distance to the south-west is the heart of one of the chief manufacturing areas of Yorkshire —the Heavy Woollen District—whilst to the south at a distance of about eight miles lies the city of Wakefield. It requires no special

TABLE I. *Location of Collecting Stations.*

Direction from Centre	Distance from Centre	Name of Locality
	miles	
N.	7	Harewood
N.	5	Alwoodley
N.	3	Meanwood
N.E.	7	Thorner*
N.E.	5	Shadwell
N.E.	3	Roundhay
E.	7	Garforth
E.	5	Manston
E.	3	Seacroft
SE.	3	Rothwell
S.	3	Middleton
S.W.	3	Gildersome
W.	3	Bramley
N.W.	3	Kirkstall

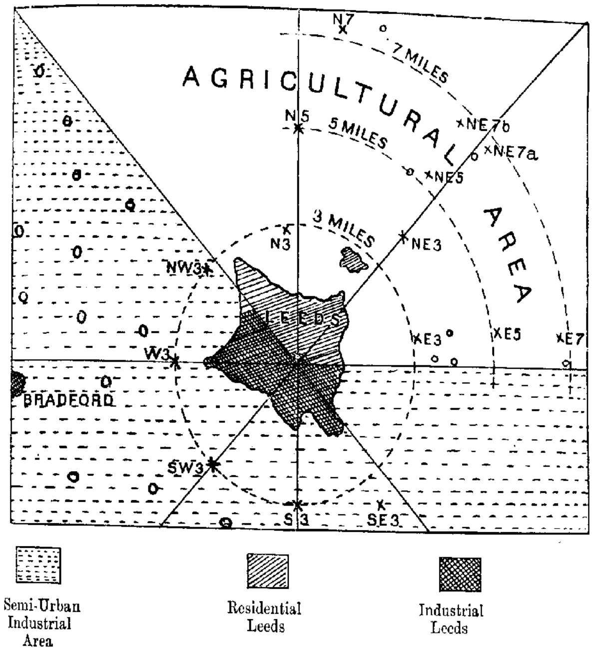
* It was necessary to abandon the collecting station first selected in this district, as all the samples of rain obtained showed unmistakable evidence of excessive pollution from the village of Thorner, which lay close by to windward of the collecting station (see Fig. 1).

investigation to demonstrate that throughout the whole of this area and much farther afield in these directions the atmosphere is universally polluted by smoke to a very appreciable extent.

On the other side of Leeds, however, there are no such complications, as the country to the north, north-east and well down towards the east is purely agricultural in character.

Any general atmospheric pollution of the character of smoke that prevails over this area may thus be ascribed with a high degree of certainty to the chimneys of Leeds or of remoter industrial centres, and should show a marked gradation at points successively more remote from the town. In selecting stations at which the funnels could be

kept conveniently under observation difficulty was experienced in some cases in evading pollution from the smoke of the neighbouring village. Reference has already been made to the case of Station N.E. 7 (*a*), and we have reason to believe that the results obtained at Stations N.E. 5 and E. 5 were also unduly high for similar reasons (Fig. 1).



○ Village × Collecting station

Scale: 1 inch = 2 miles.

Fig. 1. Showing positions of collecting stations with regard to Leeds and nature of surrounding areas.

At each station the samples were collected by means of a copper funnel, 15 inches in diameter, with vertical upper walls 3 inches deep, the funnel being supported on iron legs so that its rim was 18 or 20 inches above the surface of the ground. Under the funnel was placed a large bottle standing in a wooden box. The capacity of the bottle was 4½ litres, so that it would accommodate a rainfall of roughly 1½ inches. A scale was marked on the side of the bottle in order that

it might serve as a rain-gauge. When the bottle was nearly full, the rainfall contained in it was read off, the whole thoroughly shaken and a quantity of about two litres rapidly transferred to a clean Winchester bottle. The remainder of the rain collected was then thrown away and the large bottle replaced under the funnel.

The arrangements made for obtaining the samples in this way would have been adequate in a normal year, but were strained to the utmost by the abnormal weather conditions of 1911–12.

With the assistance available fully two days were required in order to do the round of the funnels whenever it was necessary to remove the samples, and on a few occasions rain fell so heavily and persistently that some of the collecting bottles overflowed before they could receive attention.

Further irregularities in the records occasionally arose owing to the impossibility of constant supervision of the funnels, a few samples being rejected owing to obvious undesirable pollution not of atmospheric origin, and a few other samples lost through fracture of the collecting bottles by frost, or otherwise.

In making up the totals for the year it has been necessary therefore to compute the composition of these missing samples, and this has been done by close comparison of the records of the neighbouring stations. The data assessed in this way amount to about 7 per cent. of the total number.

For these and other reasons indicated later we do not profess to have secured records of high absolute accuracy, but we regard them as amply reliable for the comparative purposes with which alone our work is concerned.

In order to ensure strict comparability of the analytical results the whole of the analyses were undertaken by one of us (D. W. S.). The range of variation in the individual samples and the probable errors of the averages are given in the Appendix, p. 408.

The following table (Table II) gives a summary of the results for the twelve months, the totals being expressed for convenience as pounds per acre. In all, 291 samples were dealt with. Twelve further samples should have been obtained, but were lost through breakage of bottles or other unavoidable cause.

No close examination of the table is necessary to discover that very marked and significant differences are shown between the various areas. It will be observed that at all the stations on the three-mile ring the pollution was high, notably in suspended matters and sulphur—the

two outstanding features of smoke pollution. Further, the more pronouncedly industrial districts (from S.E. 3 downwards) show much greater pollution than the districts equidistant from the centre of the city lying to the north and north-east.

TABLE II. *Total impurities of various kinds brought down by rain during the twelve months, July 1911—June 1912.*

(Pounds per acre)

Collecting station	Rainfall	Total suspended matters	Suspended mineral matter: Total	Suspended mineral matter: as per cent. of total susp. matter	Sulphur expressed as SO_3	Chlorine	Nitrogen
	inches	lbs.	lbs.	per cent.	lbs.	lbs.	lbs.
N. 7*	33·2	72	38	53	128	44	6·7
N. 5	33·1	104	57	55	192	59	9·1
N. 3	30·1	175	107	61	218	53	5·9
N.E. 7 (*a*)†	31·0	131	78	60	241	53	8·5
N.E. 7 (*b*)†		121	73	60	129	40	5·8
N.E. 7 (*c*)†		126	75	60	162	46	6·6
N.E. 5	29·4	150	92	61	168	43	9·1
N.E. 3	30·5	120	62	52	186	45	8·5
E. 7	29·1	122	63	51	168	43	6·6
E. 5	28·7	212	106	50	171	50	8·0
E. 3	27·8	200	109	54	207	54	7·9
S.E. 3	25·8	353	250	71	357	63	8·9
S. 3	28·2	286	154	54	269	47	9·3
S.W. 3	32·7	239	149	62	268	56	8·0
W. 3	30·4	292	178	61	284	56	8·7
N.W. 3	28·3	194	110	57	380	70	7·4

* It will be convenient to describe the stations in this fashion, "N. 7" being the station situated 7 miles to the north; "S.E. 3" the station situated 3 miles to the south-east, and similarly throughout.

† See footnote, p. 392. Station (*a*) is the station first selected in this area and abandoned on Nov. 21st, 1911, in favour of Station (*b*). The data given for (*a*) and (*b*) are computed on the assumption that the collection of samples had continued at (*a*) or (*b*) respectively for the whole of the 12 months. The data given under 7 (*c*) are the totals for the year as actually obtained.

Further, there is an obvious sharp falling-off in general pollution on passing away from the city northwards or north-eastwards, but a much more gradual fall in passing eastwards. The influence of the prevalent westerly winds in disseminating the smoke is thus brought out in very interesting fashion, although there is a certain degree of

complication in that this easterly line of stations is situated just on the northern fringe of the coal-mining area.

One further item of general interest may be singled out before passing to a more detailed consideration of the results—the relatively high degree of pollution even in the cleanest direction at a distance of seven miles from the city[1].

RAINFALL.

There was an appreciable difference in the rainfall at the different stations, the range in the yearly totals being from 26 inches at Station S.E. 3 to 33 inches at the more remote northerly stations—a variation of more than 20 per cent. The higher rainfalls were recorded on the more hilly side of the city, commencing with south-west and working round by west to north.

SUSPENDED MATTER.

It has proved almost impossible to obtain an accurate measure of the total suspended matter of atmospheric origin falling at each station. In the first place, it was soon found to be necessary to protect the funnels from birds by fixing a screen of black cotton thread over them. Further trouble was experienced at most centres by flies and other insects, fragments of leaves, etc., finding their way into the samples, and the difficulty was only partially overcome by placing discs of copper gauze of close mesh in the funnels. It is further possible that at some centres (*e.g.* S.E. 3) the funnels may have received an abnormal amount of dust owing to the proximity of roads or cultivated land.

This may to some extent account for the high proportion of mineral matter in the total suspended matters at these stations, but at the same time the fact must not be overlooked that most of the stations where the proportion of ash in the total suspended matters is above the average (56 per cent.) are close to the industrial area, where a high proportion of grit in the smoke might be expected.

[1] It is of interest to note that the proportion of impurities contained in the samples collected during the period of the great coal miners' strike (March, April, 1912) was decidedly below the normals for the periods immediately preceding and following it—especially in the industrial area. This can scarcely be regarded as a coincidence, and it is probable that in a year with normal output of smoke the yearly totals might be appreciably higher than we have found.

Despite irregularities in individual samples due to the causes above outlined, the results as they stand are sufficiently precise to bring out the marked difference between the "industrial rural" area to the south and west and the agricultural area to the north and north-east. Moreover they indicate the steady falling-off to the north with increased distance from the city, whilst to the north-east and east the falling-off is not so pronounced. The results for Stations N.E. 5 and E. 5 are abnormal in this respect, being higher than those for the stations nearer to the city, N.E. 3 and E. 3 respectively. The high proportion of ash affords an explanation in the former case, whilst at the latter station, owing to the proximity of trees and inadequate local supervision, great trouble was experienced with birds and leaves.

The grading of the stations comes out rather more sharply if made on the basis of the ash-free suspended matters, as may be seen from the following chart:—

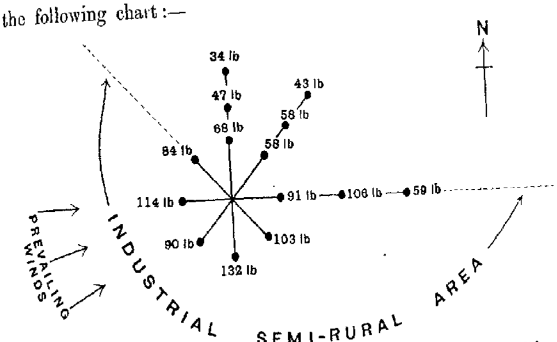


Fig. 2. Chart showing the amounts (lbs. per acre per annum) of ash-free solids brought down at various stations.

It is clear from the chart that the results in general agree closely with what might be expected from a comparison of the relative positions of the stations with regard to the smoke-distributing area.

"*Tar.*" In the majority of the samples taken during the first six months estimations were made of the ether-soluble matter ("tar") contained in the suspended matters, with results which may be summarised as follows:—

Percentage of tar in total suspended matters.

	Clean districts	Dirty districts
Maximum	15·8 (Station N. 7)	10·9 (Station W. 3)
Minimum	6·8 (,, N. 3)	4·8 (,, S.E. 3)
Average	9·5 ± ·7	7·6 ± ·2

These results are quite in harmony with expectations since in industrial districts a considerable proportion of the coal consumed is subjected to a much more thorough combustion than in rural districts, where the smoke arises mainly from domestic fires whose imperfect combustion gives rise to soots rich in tar[1].

SULPHUR.

In every sample, after removal of the suspended matters, the total sulphur was determined by precipitation with barium chloride after boiling with bromine water—the determinations being made throughout in duplicate.

The results are summarised in Table II, and are surprisingly high all round, ranging from 128 lb. at Station N. 7 to 380 lb. at Station N.W. 3. These figures are even higher than those obtained in 1907–8 at some of the stations within the city[2], although this difference is doubtless partly accounted for by the decidedly heavier rainfall of the later period.

A study of the results leaves no doubt that the sulphur-content of the rain samples is the most characteristic indication of the degree of pollution of the atmosphere with coal smoke. The lowest amount recorded at stations on the three miles radius was 186 lb., at Station N.E. 3, a quantity two-and-a-half times as great as was found in the earlier investigations for the same locality! The two sets of observations are in agreement, however, in indicating that the sulphur-content of the atmosphere at this distance from the centre of the city is lower in this north-easterly direction than in any other.

On passing further away from the city, however, the sulphur falls most rapidly in the northerly direction (218 lb. to 128 lb.), less in the north-easterly direction (186 lb. to 129 lb.), and still less towards the east (207 lb. to 168 lb.).

The totals for the stations on the industrial side of the city are uniformly high, and such differences as are recorded must be attributed largely to the varying degree of unavoidable local smoke pollution. Thus the higher figure obtained at Station N.W. 3 than at W. 3 finds an obvious explanation in the presence to the west of the former within a distance of about half a mile of large ironworks producing

[1] Cf. Crowther and Ruston, This *Journal*, IV. 38; Cohen and Ruston, *Journal Soc. Chem. Ind.* xxx. (1911), 1360.

[2] Crowther and Ruston, *loc. cit.* 34, 40.

great volumes of smoke, whilst a line of railway also runs in the near neighbourhood.

Sulphur present in forms other than Sulphate. In the majority of the samples collected during the first six months, determinations were made not only of the total sulphur, but also of the sulphur present as sulphate, the difference then representing the sulphur present in lower stages of oxidation. The amounts of rain available for these estimations were, however, too small to admit of this difference being determined with great precision. We would merely record therefore that the average of all samples indicated that 6·9 °/。 of the sulphur was present in forms other than sulphate, the averages for the clean and dirty districts being 7·6 (± ·2) and 6·0 (± ·8) per cent. respectively. Excluding doubtful data, the highest proportion (10·4 °/。) was found at Station N. 7 and the lowest (4·6 °/。) at S.E. 3.

The higher figure obtained for the relatively clean districts must be attributed to the prevalence there of smoke of domestic origin (cf. also tar, p. 397). It is possible that, contrary to the commonly held opinion, the smoke arising from the combustion of coal under industrial conditions may in some cases contain its sulphur not mainly as sulphur dioxide but almost entirely in the form of sulphate. This was brought out clearly by the investigations of Herbig, which indicated that not more than $\frac{1}{100}$ of the sulphur of the coal left the chimney as SO_2.[1]

CHLORINE.

The chlorides present in the rain samples were determined by titration with silver nitrate solution in presence of potassium chromate. The presence of traces of copper (from the funnels) masked the end-point unless a slight excess of the indicator were added and the liquid filtered before titration.

It will be seen that the range of variation between the different stations (from 40 lb. at N.E. 7 to 70 lb. at N.W. 3) was much less with regard to chlorine than in the case of sulphur. There is still evident, however, the differentiation, although not so sharp, between the industrial and the agricultural areas. There are also indications of a falling-off in the amount of chlorine on passing away from the city.

The chlorine, however, does not appear to be as reliable a guide to smoke pollution as the sulphur.

[1] W. Herbig, *Zeits. angew. Chem.* XXII. 1882.

NITROGEN.

In the earlier samples separate determinations were made of the nitrogen present as ammonia, nitrate, and in "albuminoid" form—the last-named being the nitrogen expelled as ammonia on boiling the water (freed from ammonia) with alkaline permanganate. In the later samples the three determinations were combined in one to obtain simply the total nitrogen present. The results were probably a little lower than would have been obtained by determining the three forms separately.

The yearly totals given in Table II range from 5·8 lb. (Station N.E. 7) to 9·3 lb. (Station S. 3), but there is a lack of the regularity in the gradation of the stations that is evident in the sulphur figures.

The nitrogen results showed two periods of abnormal figures, viz. at the beginning and end of the twelve months. The former we attribute to the pollution of the samples at that period (July, August, 1911) by the droppings of birds (cf. p. 396), but for the latter we cannot trace the cause, unless it be that the prevalence of severe thunderstorms during May and June, 1912, caused unusual enrichment of certain samples with nitrogen.

If the results for these two periods be eliminated the totals for the remainder of the year are as follows:

Station	N. 7	—	3·3 lbs. per acre
	N. 5	—	3·9
	N. 3	—	3·75
	N.E. 7	—	3·7
	N.E. 5	—	4·9
	N.E. 3	—	3·7
	E. 7	—	3·8
	E. 5	—	4·3
	E. 3	—	4·1
	S.E. 3	—	6·0
	S. 3	—	5·9
	S.W. 3	—	5·0
	W. 3	—	5·1
	N.W. 3	—	4·4

The difference between the agricultural and industrial areas is now more obvious, the average of the first nine stations being 3·9 lb., as against the average of 5·24 lb. for the remaining five stations, all situated in the industrial area.

Nitrogen in Different Forms.

The organic nitrogen (where estimated) ranged from 6 °/。 (Station N.W. 3) to 21 °/。 (Station S. 3) of the total nitrogen.

Nitrate (+ nitrite) nitrogen ranged from 17 % (Station S. 3) to 30·8 % (Station N.W. 3) of the total nitrogen.

Nitrogen present as ammonia ranged from 61 % (Station N. 5) to 75 % (Station N.E. 5).

The averages for all samples in which separate determinations were made were as follows:

	Percentage of total nitrogen present as		
	Ammonia	Nitrate	Organic matter
All stations	67·3	21·5	11·2
9 "clean" stations	67·7	20·7	11·6
5 "dirty" stations	66·8	22·7	10·5

The ratio of ammoniacal nitrogen to nitrate nitrogen is thus on the average of all stations 75·8 : 24·2—almost identical with the ratio obtained in the earlier Leeds investigations, the corresponding ratios for the clean and dirty districts being 76·6 : 23·4 and 74·7 : 25·3 respectively.

The proportion of organic nitrogen, it will be noted, was on the whole slightly lower in the dirty districts than in the "clean" area, an observation which agrees with the correspondingly lower proportion of sulphur in lower stages of oxidation (p. 399).

FREE ACID.

The samples of rain collected by Crowther and Ruston in Leeds and at Garforth (Station E. 7 of present series) were in many cases distinctly acid to methyl orange.

The reaction of each sample of the present series has therefore been carefully noted, but in only the following cases was an acid reaction obtained:

Station	Total samples	No. of acid samples
N. 3	23	1
E. 7	20	2
S.E. 3	23	2
S. 3	23	3
S.W. 3	20	1
W. 3	21	2
N.W. 3	23	2
Total	153	13

Of the 140 non-acid samples, some were neutral, but most were distinctly alkaline to methyl orange, congo red and litmus.

It has not been possible to obtain a reliable measure of the free acids contained in the rain, owing to their action upon the copper

of the funnel. An attempt was made to get over the difficulty by applying a coat of lacquer to the copper, but it did not prove successful. The neutralisation of acid by the copper was demonstrated by comparisons made at Garforth between rain collected by means of a glass funnel and that collected through the copper funnel. In the two cases where acid samples were obtained through the copper funnel, the rain collected by the glass funnel was distinctly more acid, whilst on two further occasions the "glass funnel samples" were acid, whilst the corresponding "copper funnel samples" were not.

A further difficulty in the way of obtaining reliable determinations of the acidity of the rain lay in its action upon the glass of the collecting bottle. Although the inside of the bottle was soaked in hydrochloric acid for some time and then thoroughly washed before use, tests with waters of known acidity indicated a decrease of acidity during storage. In one case, where the two halves of such a water were put in separate untreated bottles and left overnight, a very marked difference was found on the following morning. There can be little point therefore in recording the amounts of acid actually found in the samples showing acid reaction, as they must have been much below the original acidity of the rain at the time of falling upon the funnels. It is certain that with more satisfactory arrangements for the collection of the samples the presence of free acid would have been more frequently detected.

EFFECTS OF SMOKE UPON VEGETATION[1].

In the previous paper brief reference was made to the effects of smoke pollution in accelerating the fall of leaves from trees. More extensive observations with regard to this and other effects have now been made throughout the area under investigation, and the general results may be briefly summarised.

No general effect upon the opening of the buds of trees could be detected. The differences between trees of the one species in any one district were quite as great as those found between different districts.

The majority of the trees round about the town were almost in full leaf by the beginning of May, but by May 29th smoke damage began to be evident within two miles of the centre of the city by the

[1] For an exhaustive discussion of the most important work on the subject up to 1903 see Haselhoff and Lindau, "Die Beschädigung der Vegetation durch Rauch" (*Gebr. Bornträger*, Leipzig, 1903).

appearance of the leaves—sycamores and limes in particular showing characteristic brown blotches.

On May 30th observations were made in extensive woods near Station S. 3 (Middleton), flanked on the south-east by large collieries and coke-ovens. Very little leaf-staining was found inside the woods, but along the edge nearest to the works there were abundant signs of damage. Young ash leaves were found shrivelled up and blackened. Elms and sycamores were also badly marked. A few thin-leaved sycamores, growing somewhat overshadowed by others, had their leaves hanging limp and yellow, and the edges had disappeared, leaving a rough discoloured fringe.

Throughout the district from Station W. 3 (Bramley) to Station N.W. 3 (Kirkstall) there was at this early period abundant evidence of damage—beech, oak and sycamore showing brown leaf-blotching: birch had brown curled-in edges and elm black shrivelled edges. The damage was intensified in the latter district, owing to the presence of a large forge (cf. p. 398), and sycamore, elm, oak, laburnum, beech, chestnut and others showed leaf damage.

Similarly, at the other end of the more polluted area, near Station S.E. 3 (Rothwell), tree leaves in general were badly stained and often shrivelled up.

Thus, throughout the polluted area to the south and west of Leeds a considerable proportion of the leaves of trees were badly damaged before they had been expanded one month.

Throughout the cleaner districts to the north and north-east no appreciable amount of leaf-damage could be detected at this period.

During June matters became considerably worse throughout the polluted area, and in the case of large numbers of trees the leaves can have been of but little use. For such trees the annual period of growth is thus very short and their progress correspondingly slow. This was confirmed by the examination of sections of oak trees cut down in the woods above-mentioned. The annual rings were found to be very narrow[1].

With regard to the general comparison of the clean and smoky districts the outstanding features are the small numbers of trees, and indeed the total absence of coniferous trees throughout the latter districts, whereas in the cleaner districts the trees are much larger and carry more and bigger leaves.

1 Cf. Haselhoff and Lindau, *loc. cit.* 106.

Great differences are found between individual trees of the same species with respect to their powers of resistance against smoke. Even in the polluted districts it is only trees here and there that are dead or have dead tops, branches or twigs. Ash, poplar, apple, elm, beech, and particularly oak and birch, were found thus permanently damaged. Conifers, however, are entirely absent, despite attempts to introduce them. In the cleaner districts Black Austrian pine, Scots pine and spruce were found between Station N.W. 3 (Kirkstall) and N. 3 (Meanwood); pines at Meanwood (N. 3); cedars and several species of pine at Gledhow (N.E. 3); Austrian and Scots pine at Roundhay (N.E. 3); whilst at Seacroft (E. 3) cedars, Scots and Austrian pine grew, but larch and spruce had failed. In one part of this district some large larch trees were found in a sheltered valley, but were said to have made no growth during the last generation, but rather to be "going back owing to the smòke" and hence are to be cut down shortly.

Passing further away from the town, larch—too sensitive nearer the city—was found five miles out to the north-east (Shadwell), whilst at Garforth (E. 7), despite local coalmines, Scots, Austrian and other pines are represented, also spruce and larch.

The observations were extended to other classes of vegetation, especially agricultural crops; but these will be dealt with in a subsequent paper, along with the results of further investigations that are in progress.

It has been clearly demonstrated by several investigators that one great cause of the damage inflicted by smoke is the presence in it of sulphur dioxide. This penetrates the leaf and is retained there, so that in general leaves from smoke-polluted districts are richer in sulphur than similar leaves from clean districts. The sulphuric acid of the smoke will have much the same effect. Hence the sulphur-content of leaves should furnish guidance in the diagnosis of smoke-pollution, and indeed is now generally accepted as one of the most reliable aids to such diagnosis[1].

In order to test this, samples of leaves (sycamore) were taken at the end of July 1911—after a month of dry weather—in each of four districts[2]—two dirty (S.W. 3 and S. 3) and two clean (N. 3 and N.E. 3). Each sample was carefully reduced to ash and the total

[1] Haselhoff and Lindau, *loc. cit.* 57.

[2] All on one geological formation, viz. the coal measures. The risk of complications arising from differences of soil was thereby probably reduced.

sulphate in the ash then determined (as $BaSO_4$). The results are summarised below, together with the amounts of sulphur in the rain collected during the period July 1 to August 23 and the totals for twelve months (cf. p. 395).

District	SO_3 in dry matter* %	Total S. in rain July 1—Aug. 23 % SO_3	Total S. in rain (lbs. per acre per ann.) SO_3
S.W. 3	1·36	14·6	268
S. 3	1·11	13·3	269
N. 3	0·91	10·7	218
N.E. 3	0·55	10·0	186

In order to ascertain how much of the sulphate was on the outsides of the leaves, other samples were taken at S. 3 and N. 3 and dipped in distilled water six times before drying. The percentages of SO_3 in the ash were then found to be reduced to ·89 and ·75 respectively—results, however, of only a single determination in each case.

In the following year the tests were repeated with greater precautions in the choice of samples and at different periods.

On May 29th, 1912, when leaf-blotching was first noticed, samples of 50 sycamore leaves were taken in each of four districts, the leaves being taken from a number of trees in each case. In one district (S. 3) duplicate samples were taken. In all cases leaves of about the same size were selected.

On August 13th, after a period of continuous heavy rain, another set of samples was taken. In each case determinations were made of the total sulphur, water-soluble sulphur, and ash in the leaves.

Percentage in dry matter.

District	Total SO_3		Water-sol. SO_3		Chlorine		Total ash		Condition of leaves	
	May	Aug.	May	Aug.	May	Aug.	May	Aug.	May	Aug.
N. 3 (clean)	·48	·75	·14	·48	·63*	·94*	6·8*	7·8*	Healthy	Slightly damaged
N.E. 3 (clean)	·54	·86	·29	·62	·63*	·91	4·9*	7·8	,,	,,
N.W. 3 (dirty)	·72	·83	·39	·69	·75	—	5·2*	8·0*	Damaged	Had been practically killed for some time
S. 3 (very dirty) (1st sample)	·79	·86	·56	·80	—	—	5·3*	8·6*	,,	
S. 3 (2nd sample)	·76	·85	·48	·83	—	—	—	—	,,	

* Single analysis only.

It will be noted that in the young leaves (May) the total sulphur showed a marked difference between the clean and dirty areas, but that the differences were appreciably less at the later stage of growth. The water-soluble (inorganic) sulphur, however, showed very pronounced differences at both stages.

It is commonly stated that smoke-damaged leaves contain a higher proportion of ash than normal leaves, and the data obtained in the cases quoted above support this view. They are too meagre, however, to serve as a basis for discussion, as are also the data for chlorine.

One further set of observations may be recorded since they bear out previous observations which have received some little criticism. In the previous paper the opinion, based upon microscopical examination of leaf-sections, was expressed that the solid matters of smoke might cause an actual clogging of the stomatal openings of leaves. Further examinations have recently been made by a different observer (D. W. S.) with leaves of evergreens taken on February 14th, 1913, after two days' dense fog. In each case a thin slice was skimmed off from the under surface of the leaf and subjected to microscopic examination. In the case of aucuba the soot occupied the stomatal openings on many preparations. Similarly with privet, the soot had gathered in the openings and also followed the lines of the guard cells. In the case of holly and ivy the soot was generally distributed, and showed no decided preference for the stomatal openings, although a few were occupied. The matter on the leaves and lodged in the stomatal openings where present was quite sharply defined and opaque.

SUMMARY.

The rain analyses summarised in this paper bring out very clearly the marked difference in the purity of the atmosphere between the industrial-rural area to the west and south and the purely agricultural area to the north and north-east of Leeds.

They show that the sulphur-content of the rain falling at a given station affords a fairly reliable diagnosis of the degree of pollution of the atmosphere by smoke, provided the observations be prolonged over several months. Further, evidence has been obtained in confirmation of that adduced by earlier observers, that the sulphur-content of the leaves of trees may afford useful assistance in the diagnosis of smoke-pollution. In preliminary tests the proportion of sulphur present in

the leaf as sulphate gave a sharper grading of the pollution in different districts than the total sulphur.

The rain analyses show further that appreciable smoke-pollution still remains throughout the agricultural area at distances of seven miles from the city, the rate of improvement of the atmosphere on passing away from the city into the purely agricultural areas being appreciably slower in the direction of the prevailing winds than in other directions.

In a general way the analyses tend to show that the smoke, though in greater quantity, is in a higher state of oxidation in the dirtier or more industrial districts—due to more efficient combustion of the coal.

Throughout the industrial-rural area abundant evidence of damage to leaves was found, and there can be no doubt of the consequent check to growth.

The investigations summarised in this paper have been rendered possible by a grant from the Board of Agriculture and Fisheries from funds placed at their disposal by the Development Commissioners, and we desire to express our appreciation of the opportunity thus afforded of carrying out the work which previous investigations indicated as being desirable.

We would also here acknowledge our indebtedness to all those who have granted us facilities for the collection of samples at the different centres, and to our colleague, Mr A. G. Ruston, B.A., B.Sc., for assistance in various ways.

Further experiments are in progress, by means of which we hope to get a direct measure of smoke damage upon agricultural crops in semi-urban areas, and also further criteria for its diagnosis.

APPENDIX.

Table showing range of variation in individual samples of rain, mean composition and probable error of mean for each station.

(Parts per 100,000.)

	Total suspended matter	Ash in suspended matter	Chlorine	Total sulphur (expressed as SO_3)	Total nitrogen
Station N. 7					
Max. ...	2·66	1·53	3·31	3·63	0 187
Min. ...	0·04	0·03	0·15	0·37	0·034
Mean ...	0·96 ± ·12	0·51 ± ·07	0·58 ± ·13	1·70 ± ·14	0·089 ± ·007
Station N. 5					
Max. ...	3·50	2·38	5·84	6·08	0·235
Min. ...	0·36	0·10	0·25	0·59	0·037
Mean ...	1·38 ± ·12	0·76 ± ·10	0·79 ± ·18	2·56 ± ·23	0·121 ± ·009
Station N. 3					
Max. ...	7·00	5·20	3·87	7·12	0·160
Min. ...	0·40	0·17	0·20	0·74	0·037
Mean ...	2·57 ± ·23	1·57 ± ·19	0·78 ± ·11	3 19 ± 0·25	0·086 ± ·006
Station N.E. 7 (*c*)					
Max. ...	4·76	3·09	3·57	5·87	0·234
Min. ...	0·32	0·07	0·15	0·69	0·031
Mean ...	1·79 ± ·21	1·07 ± ·14	0·65 ± ·12	2·30 ± ·22	0·094 ± ·010
Station N.E. 5					
Max. ...	7·74	5·51	3·55	5·54	0·246
Min. ...	0·20	0·12	0·10	0·64	0·046
Mean ...	2·26 ± ·35	1·39 ± ·26	0·64 ± ·13	2·52 ± ·24	0·137 ± ·010
Station N.E. 3					
Max. ...	3·97	2·50	2·63	6·62	0·364
Min. ...	0·20	0·06	0·30	0·67	0·055
Mean ...	1·73 ± ·17	0·90 ± ·12	0·65 ± ·08	2·70 ± ·27	0·123 ± ·013
Station E. 7					
Max. ...	5·88	4·31	3·10	6·95	0·278
Min. ...	0·54	0·13	0·10	0·58	0·052
Mean ...	1·85 ± ·25	0·95 ± ·20	0·65 ± ·10	2·54 ± ·27	0·100 ± ·008
Station E. 5					
Max. ...	11·13	4·47	3·82	7·16	0·302
Min. ...	0·68	0·42	0·10	0·74	0·027
Mean ...	3·26 ± ·40	1·64 ± ·20	0·76 ± ·13	2·63 ± ·23*	0·123 ± ·009
Station E. 3					
Max. ...	12·54	6·02	2·57	12·54	0·455
Min. ...	0·99	0·44	0·40	0·88	0·052
Mean ...	3·18 ± ·41	1·73 ± ·28	0·86 ± ·09	3·29 ± ·51	0·126 ± ·020
Station S.E. 3					
Max. ...	22·82	17·47	3·30	12·85	0·492
Min. ...	1·03	0·74	0·30	2·16	0·049
Mean ...	6·04 ± ·83	4·28 ± ·69	1·08 ± ·10	6·10 ± ·45	0·152 ± ·014
Station S. 3					
Max. ...	17·28	9·98	2·88	8·78	0·351
Min. ...	1·52	0·66	0·20	1·34	0·065
Mean ...	4·48 ± ·51	2·42 ± ·32	0·74 ± ·10	4·22 ± ·26	0·146 ± ·009
Station S.W. 3					
Max. ...	6·77	5·11	2·85	8·59	0·193
Min. ...	0·65	0·57	0·27	0·74	0·053
Mean ...	3·23 ± ·31	2·01 ± ·22	0·75 ± ·09	3·61 ± ·34	0·107 ± ·006
Station W. 3					
Max. ...	12·21	9·15	3·10	8·45	0·283
Min. ...	0·97	0·40	0·25	0·89	0·046
Mean ...	4·24 ± ·44	2·59 ± ·36	0·82 ± ·08	4·13 ± ·28	0·126 ± ·009
Station N.W. 3					
Max. ...	7·79	5·12	2·25	12·39	0·354
Min. ...	0·90	0·46	0·30	1·65	0·050
Mean ...	3·03 ± ·25	1·72 ± ·17	1·09 ± ·08	5·90 ± ·46	0·116 ± ·010

CYANOGENESIS UNDER DIGESTIVE CONDITIONS.

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THE considerable amount of attention paid, during recent years, to the liberation of hydrocyanic acid from plant products has caused grave suspicion to be cast upon certain of the latter which are commonly used for feeding purposes. Of the food-stuffs which produce hydrocyanic acid in considerable quantity on maceration with water, the most important are, without doubt, linseed and linseed cake, Java beans and other varieties of *Phaseolus lunatus*, and immature great millet (*Sorghum vulgare*). As is well known, definite cases of cattle-poisoning have been traced, in numerous instances, to the two last-named commodities, and linseed cake has also come in for a certain amount of suspicion, certain cases of poisoning amongst stock having been attributed to its cyanogenetic properties.

In all cases of prussic acid-forming feeding-stuffs so far examined the hydrocyanic acid is liberated by the hydrolysis of a glucoside by a co-existent enzyme, when the ground plant or seed is placed in water. The generally innocuous character of linseed cake was first explained by Henry and Auld [1] and by Dunstan [2] as being due to the destruction of the glucosidoclastic enzyme present in the seed during the hot process of expressing the oil. Further investigation by the author [3, 4] has shown that this view cannot be maintained, since the majority of linseed cakes produce hydrocyanic acid on maceration with water, the amounts varying from 0·001 per cent. to 0·052 per cent., and only in a few cases is no HCN formed, owing to the enzyme having been destroyed. On incubation at temperatures approximating to blood-heat half the available prussic acid is sometimes liberated in fifteen minutes and the maximum practically attained within six hours. Despite this fact, sheep fed with linseed cake containing 0·052 per cent.

of available HCN, in quantities up to 4 lbs. per day (5 lbs. per 100 lbs. live-weight), suffered no ill effects, although the proportion of available hydrocyanic acid far exceeded the lethal dose as determined by direct experiment with potassium cyanide (Auld, *loc. cit.*). Investigation has shown that the enzymic hydrolysis of the cyanogenetic glucoside is greatly inhibited by nearly all the conditions prevailing in the digestive tract. Acidity and alkalinity both have a strongly retarding influence on the rate of evolution of prussic acid, as also have the digestive enzymes themselves. Many of the feeding adjuncts have a strongly inhibiting action on cyanogenesis; salt, glucose and molasses are strongly active in this direction, as is also cellulose. The effect of the last named is most profound and was discovered by incubating ground linseed cake with many of the common fodders which are likely to be fed with it. Some of these fodders contain enzymes capable of hydrolysing sugars, glucosides, etc., but despite this fact the rate of evolution of hydrocyanic acid from linseed cake is invariably retarded thereby.

Influence of Cellulose on Cyanogenesis.

The fodders initially used in these experiments were mostly grown specially for the purpose, the green plants being dried at ordinary temperature, disintegrated and reduced to powder in the mill. In each case observations were made both with the powdered plant and with extracts prepared from it. 25 grams of cake were used in each experiment. The plant extracts were obtained by digesting the ground material with twelve volumes of water containing a few drops of toluene for 48 hours at 38 degrees C. The time of incubation was 30 minutes in each case.

The action of the various plant extracts, either in a positive or negative direction, is very small and is not to be correlated with the existence in them either of active enzymes (as in the case of rye, tares and maize) or of inhibiting substances like sugar. The inhibiting action of the ground plant on the other hand is considerable and invariable. That the effect is due to the cellulose was proved in the case of Helianti, a very coarse fodder. Fifteen grams of this material were ground and extracted successively with ether, alcohol and water. The various extracts and the exhausted residue were then incubated separately with 25 grams of linseed cake for 30 minutes at 35 degrees C.

TABLE I.

Plant	Weight of ground plant	Volume of extract	HCN formed	Temperature of action	Control HCN formed
	gram	c.c.	%	deg. C.	%
1. COMMON RYE. *Secale cereale* L. Just before flowering	— 15	50 —	0·032 0·022	38·0	0·030
2. COMMON VETCH OR TARES (Variety Winter). *Vicia sativa*, L. Cut in full flower	— 15	100 —	0·031 0·028	37·5	0·031
3. LUCERNE. *Medicago sativa* L. Flowers unopened	— 20	100 —	0·030 0·026	37·5	0·031
4. SAINFOIN (Variety Giant). *Onobrychis sativa*, Lam. In full bloom	— 15	100 —	0·029 0·025	37·5	0·031
5. KIDNEY VETCH. *Anthyllis vulneraria*, L. Height 10 inches	— 15	75 —	0·028 0·024	37·5	0·030
6. HELIANTI. *Helianthus macrophyllus.* Height 2 ft.	— 15 20	100 — —	0·018 0·013 0·011	38·0	0·026
7. MAIZE. *Zea Mais.* Height 4 inches	— 20	100 —	0·021 0·019	38·0	0·026
8. MAIZE. Height 30 inches	— 15	100 —	0·021 0·017	38·0	0·026

Material used	HCN formed
Cake alone	0·021 per cent.
Cake + ether extract	0·019 ,, ,,
Cake + alcohol extract	0·017 ,, ,,
Cake + aqueous extract	0·019 ,, ,,
Cake + extracted residue......	0·010 ,, ,,

Cellulose was prepared in a fine, disintegrated condition by digesting Swedish filter paper, cut into small pieces, successively with 2 per cent. sulphuric acid and 2 per cent. caustic soda. The addition, in this form, of cellulose to linseed cake depressed the formation of prussic acid in a similar manner to the fodders. A bulkier cellulose prepared from cotton wool had a similar effect. Experiments with salicin and amygdalin showed conclusively that this action is due to the adsorption of the cyanogenetic enzyme by the cellulose. As these experiments are being continued on other lines, they will not be quoted further here. It is sufficient to point out the profound inhibiting action of the fibrous feeding-stuffs on cyanogenesis from linseed cake under digestive conditions.

Effect of Acids and Alkalies under Digestive Conditions.

It is well known that most glucosidoclastic enzyme actions are strongly inhibited by dilute acids and alkalies, although mere traces of acid and base are occasionally helpful. In face of this knowledge, a statement by Lander [5] that "fermentation" (*i.e.* cyanogenesis) "goes on in 1 per cent. hydrochloric acid and also in 1 per cent. sodium bicarbonate solutions, and would not therefore be inhibited by the body fluids," appears remarkable, and is, as a matter of fact, incorrect. These concentrations are equivalent roughly to N/4 HCl and N/12 $NaHCO_3$.

A communication by Collins [6] lays especial stress upon the effect of acidity, and points out that a linseed cake acidified to reproduce digestive conditions (about N/18 HCl) fails to evolve hydrocyanic acid. Even acids of one-hundredth normal strength prevent the enzyme working, and hydrochloric acid of one-thousandth normal strength produces a slackening in the rate of evolution. The effect of acidity is certainly profound, though the author has found that one-hundredth normal hydrochloric acid may still allow a certain amount of cyanogenesis to take place under certain conditions. In the experiments described by Collins one gram only of linseed was used for each experiment (*loc. cit.* p. 100) and the HCN formed was continually removed by a current of inert gas. The experimental temperature used was 45 degrees C. which is above the optimum temperature of phaseolunatase [7]. Working in closed vessels at 37·5 degrees C. with 20 grams of linseed cake for each experiment, the results quoted in Table II have been obtained.

Collins explains the generally innocuous character of linseed and linseed cake as due to this inhibiting action of acids. Indeed he says "Under normal circumstances, therefore, linseed cannot produce hydrocyanic acid when fed to carnivorous or herbivorous animals, because the acid present in the stomach prevents the enzyme in the linseed from acting," and later "Should a linseed, rich in cyanogenetic glucosides, be fed to a beast suffering from indigestion, of such a peculiar character that the food was not rendered acid, then prussic acid poisoning might set in." In making these statements Collins overlooks the general course of the food during intra-corporeal digestion. During mastication the food is mixed with the alkaline salivary juices before swallowing. In man the material is stored, without movement [8], for a period probably of about half-an-hour, in the fundus or non-acid

secreting portion of the stomach. In ruminants the food is stored in the rumen for a still longer period, and I have traced the greater

TABLE II.

Strength of acid	Time of action	HCN formed	HCN formed	Extent of Cyanogenesis
	hours	gram	%	%
N/100	1·5	0·00039	0·0019	4·7
	17	0·00078	0·0039	9·7
N/200	0·5	0·00078	0·0039	9·7
	1	0·00104	0·0052	13·0
	6	0·00259	0·0129	32·2
	12	0·00281	0·0140	35·0
N/500	1	0·00142	0·0071	17·7
	3	0·00252	0·0126	31·5
	6	0·00363	0·0181	45·2
	12	0·00415	0·0207	51·7
Neutral	0·5	0·00160	0·0080	20·0
	1	0·00254	0·0127	31·7
	3	0·00437	0·0218	54·5
	6	0·00570	0·0280	70·0
	14	0·00777	0·0388	—

portion of a meal in the paunch two hours after feeding. The regurgitated and remasticated food then passes through the reticulum and omasum before it reaches the true acid secreting stomach. There is thus hardly any question of acid inhibition of the cyanogenesis, since

TABLE III.

20 grams of cake in 150 c.c. of solution for each experiment. Temp. = 37·5° C.

Strength of alkali	Time of action	HCN formed	HCN formed	Decomposition
	hours	gram.	%	%
N/100	1	0·00098	0·0049	12·2
	3	0·00168	0·0084	21·0
	6	0·00189	0·0094	23·5
	10	0·00220	0·0110	27·5
N/200	0·5	0·00067	0·0034	8·5
	1	0·00155	0·0077	19·2
	2	0·00186	0·0093	23·2
	5	0·00264	0·0132	33·0
	12	0·00310	0·0155	38·7
N/500	35 min.	0·00132	0·0066	16·5
	1 hour	0·00173	0·0086	21·5
	3	0·00285	0·0142	35·5
	5	0·00331	0·0165	41·2
	12	0·00427	0·0213	53·2

the contents of the paunch are almost invariably alkaline, and the honeycomb and manyplies generally alkaline or neutral.

What inhibition does take place is probably due to the alkaline saliva. The average alkalinity of human saliva as measured in a number of cases is N/60. That of ruminants is of the same order. The production of hydrocyanic acid from linseed is practically completely stopped by sodium hydroxide solution of this strength although, taking it altogether, alkali seems to be rather less active as an inhibitant than acid. (Table III.)

In order to trace the extent and orientation of cyanogenesis in the animal body, sheep were fed with linseed cake before being slaughtered, and the contents of the stomach examined. Through the kindness of Mr W. M. Colebrook of Reading, to whom the writer wishes to express his thanks, three Hampshire-Down tegs were set apart for this experiment and fed as follows:

No. 1. Given 1 lb. crushed linseed cake 2 hours before killing.

No. 2. Given 1 lb. crushed linseed cake and a few handfuls of hay 2 hours before killing.

No. 3. Given 1 lb. crushed linseed cake ½ hour before killing.

The linseed cake employed was the same as that used in the previous experiment (*vid. sup.*). After the animals were killed the stomachs were removed and the contents washed into large flasks and steam distilled. The distillates were made up to known volume, tested qualitatively for HCN by the Prussian blue test and the amount formed estimated by titration with iodine in the usual manner. In each case the paunch contents were alkaline towards litmus paper, and the two intermediate stomach compartments either neutral or faintly alkaline. In every case, also, by far the greater part of the meal was still in the rumen. In the case of sheep No. 3, this applied almost wholly. The amounts of hydrocyanic acid traced throughout are given in the following table:

TABLE IV.

Sheep	Hydrocyanic acid formed, gram					Percentage of "available" HCN actually formed
	Rumen	Reticulum	Omasum	Abomasum	Total	
No. 1	0·03056	0·00388	0·00142	0·00077	0·03663	20·1
No. 2	0·02357	0·00311	0·00160	0·00065	0·02893	15·9
No. 3	0·00906	Traces	—	—	0·00906	4·9

It may be gathered from the above figures that a certain amount of prussic acid generation will usually take place from cyanogenetic feeding-stuffs when eaten by animals; also that the greater part of this formation will take place in the paunch in the case of ruminants, and in the fundus portion of the stomach in other animals. The chief limiting factor would appear to be the alkalinity of the masticated food, since the acidity of the true stomach is sufficient completely to inactivise the enzyme. This is also assisted, secondarily, by the cellulose present in the ration, and by many of the other food components.

Conditions of Possible Poisoning by Cyanogenetic Feeding-Stuffs.

The nature and alkalinity of the saliva is known to vary considerably, but even in the conditions most favourable for cyanogenesis it is very doubtful whether the alkali concentration will fall sufficiently low to allow prussic acid to be formed in large enough quantity to produce toxic symptoms. Much more important is the possibility of feeding-stuffs being used which contain free acids or which may undergo acid fermentation in the animal's body. It is notorious that fresh grass is strongly acid, and Collins (*loc. cit.*) gives its average acidity as of the order N/20. Ensilage, root tops and many other foods are distinctly acid in character and the use of such materials is likely to neutralise the salivary alkalinity and render the rumen contents neutral or acid. In the former case the use of much linseed cake containing large quantities of available prussic acid may be fraught with danger. Fortuitous neutralisation of the masticated food in this way and the feeding of improperly made linseed gruel (Auld [4]) are probably responsible for all the cases of linseed poisoning which occur from time to time.

In this connection it is interesting to review the three principal cyanogenetic feeding-stuffs already enumerated. As shown above, linseed is likely to be dangerous only in certain circumstances. Immature sorghum is especially dangerous, yet it contains little more prussic acid than many linseed cakes. The reason for its toxic character is probably its natural acidity when fed in the green state. It is possible that if it were used as soilage and fed with ground chalk, in order partially to neutralise the acid, that young sorghum would be less dangerous.

The case of *Phaseolus lunatus* is rather different. On the theory elaborated above it should be governed by the same conditions as linseed cake, despite the fact that it contains a higher proportion of active enzyme. So many cases are known however of Java beans having caused poisoning after being boiled [9, 10], whereby the enzyme is destroyed, that one is bound to reconsider the position with regard to this notoriously poisonous food-stuff. The author has shown (*loc. cit.*) that neither phaseolunatin nor amygdalin is hydrolysed in the animal's body, and the circumstances in general are so much against prussic acid formation that it seems possible that another and unsuspected poison may be present in the seeds of the varieties of *Phaseolus lunatus*. If this is so it would go a long way to clear up the question of cyanogenesis in feeding-stuffs, interesting and important alike to toxicologist and agriculturist.

The significance of prussic acid formation is also worthy of consideration from the point of view of the nutritionist. Small quantities are undoubtedly formed from certain food-stuffs in the animal's alimentary canal and it may be that this amount is distinctly beneficial. This view was originally broached in a private discussion with Dr Bernard Dyer several years back, but it is recent investigation only which has shown the likelihood of its being true. Prussic acid in small quantities is well known as a "tonic." Its formation to a limited extent in the case of linseed cake, which is inimitable for fattening purposes and for "finishing off," may be mere coincidence, but it is significant that hydrocyanic acid in other circumstances acts as a hormone, and it may possibly have a stimulating effect on the secretion of digestive juices or the activity of the lacteals and capillaries in the process of resorption. This is a point which may repay attention and the author is already following it up.

Summary and Conclusions.

1. Under digestive conditions cyanogenesis is likely to be inhibited by acids and alkalies, digestive juices, cellulose, glucose, and molasses, salt and many other feeding-stuff constituents and adjuncts.

2. Owing to the time the food remains in the digestive tract before coming to the true stomach or the acid secreting portion of the stomach, normal inhibition is caused by the alkaline character of the salivary juices. This is likely to be the chief cause of the innocuous character of linseed cake.

3. In the case of sheep fed with linseed cake shortly before being killed small amounts of hydrocyanic acid were to be found, chiefly in the rumen.

4. Cyanogenetic feeding-stuffs are most likely to be poisonous when fed with acid containing or acid producing food-stuffs, or where the hydrocyanic acid is pre-formed, as in the case of an improperly made linseed gruel.

5. The small quantities of hydrocyanic acid normally produced from cyanogenetic feeding-stuffs may possibly have a strongly beneficial action.

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ON OVARIOTOMY IN SOWS; WITH OBSERVATIONS ON THE MAMMARY GLANDS AND INTERNAL GENITAL ORGANS.

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PART II.

IN a former paper[1] evidence was adduced that the black pigment frequently found in the mammary tissue of sows is not associated (at any rate directly) with the occurrence of heat. This evidence has since been supplemented by an examination of ten strips of bacon cut from the mammary region of ten different sows. These "belly pieces" were kindly sent to us by Messrs C. & T. Harris & Co. of Calne, to whom we are indebted. These pieces were taken in the ordinary course of business, in the belief that they were cut from sows on heat. All the strips were much discoloured by large quantities of black pigment, but only four of them showed any indication of increased vascularisation. These four were to some extent congested in the close neighbourhood of the mammary ducts, and were probably obtained from sows that were on heat at the time of killing; the other six showed no such indications.

That the existence of black pigment in the mammary region is not related (at any rate directly) to the occurrence of heat is further proved by observations upon young Berkshire pigs, which had never been in use. In eight of these, four of which were spayed, and four "open," mammary pigment in some abundance was found to occur. In four others, however (two "open" and two spayed), no pigment was found excepting for a minute trace in one.

Another Berkshire sow was killed in young, and one of the foetuses was preserved by Mr John Hammond, Government Research Scholar

[1] This *Journal*, vol. IV. June 1912.

under the Board of Agriculture, who made histological preparations from the mammary region. Mr Hammond has kindly written for us the following account of his observations on this foetus, together with a note concerning his examination of a 7 weeks old pigling:

"The foetus in question (being out of a Berkshire sow showing no black pigment in her mammary glands) was examined for pigment. Sections were cut through the mammary region of the skin, and definite melanin granules were found in the rete mucosum. Further, the granules could be traced in places where the mucosum was seen dipping down to form the glands. In Fig. 1 the particles are shown to be following the course of the milk ducts, where at this stage they may be observed penetrating the dermis.

"Sections were also cut from the glands of a 'Large-Black' sow 7 weeks old. These show the pigment granules in the lower layers of cells in the rete mucosum of the skin, the colour being more intense at the bottom of the papillae. The hair follicles also showed black granules, being more intensely pigmented than the skin. In this case again the pigment granules could be seen following the course of the ducts from the nipple downwards to the gland. In the cells of the milk ducts the pigmentation was not so intense as in the cells of the glandular portion. These facts suggest that the pigment is a product of the metabolic activity of these cells, and that it is formed in greatest quantities where active growth is taking place.

"It is possible that the cells containing these granules increase at puberty and during pregnancy, or at such other times as the mammary gland hypertrophies, and so give rise to the pigmented condition which is seen in many cases over large areas in the mammary region of the adult sow."

If Mr Hammond's suggestion should prove to be correct, it provides an additional reason for spaying to those already put forward. This is a matter now under investigation.

It is interesting to note that whereas the foetuses (or at any rate one of them) contained mammary pigment, none was to be found in the pregnant mother. The non-occurrence of pigment in the mammary tissue either in this sow or in the piglings referred to above, indicates the possibility of breeding strains of Berkshire or of other coloured pigs in which there would be no melanic pigment in the bacon cut from the mammary region. The manner in which the presence or absence of this pigment is inherited is a point which requires investigation.

Imperfect Spaying.

In our former paper we gave expression to the suspicion that faulty operating was not altogether uncommon in the ordinary process of spaying sows for commercial purposes. This opinion we based largely on the fact, so frequently asserted, that some spayed sows behaved as though they were "open." We are now in a position to give definite information upon this point.

Through the courtesy of Mr Morris Wright of Whitton, Ipswich, who buys largely in the Ipswich district, two young sows were procured for us. They were both said to have been operated upon, but nevertheless were found to be behaving as if "open." These two sows (A and B) were under observation at the University farm for five and eight weeks respectively, and were found to behave as follows:

Case A showed signs of typical oestrus recurring at the normal intervals. At one of these periods this sow was put with the boar, and after considerable trouble, though possibly not more so than often happens with a normal sow mated for the first time, copulation took place. Six days after this service the sow was slaughtered and her viscera examined. The left ovary, the left Fallopian tube and the left half of the uterus were found to be present normally developed (Fig. 4). Numerous Graafian follicles in various stages of maturation were seen in the ovary, while the horn of the uterus had obviously undergone great hypertrophy since the date of the operation. No trace was found either of the right ovary, tube or uterine horn, which had evidently been removed completely. The position of junction of the right horn with the left was indicated by a scar, and there was a trace of the original wound made at the time of operation on the outside skin of the body.

Case B. Oestrus in this case was much less definite than in the former. Though the general appearance was unmistakable, swelling and congestion were less pronounced, the period was much lengthened, and there seemed to be no certainty about the date of recurrence. Notwithstanding that she was put with the boar on several consecutive days on which she showed signs of heat, in fact every day until all signs had disappeared, she resolutely refused his attentions. Some days after visiting the boar this sow was slaughtered and her viscera examined. The uterus was entirely absent. The right ovary also had evidently been completely extirpated along with the right Fallopian tube, but the left ovary with ripening follicles and large cysts (possibly formed from follicles) was found to be present (Fig. 5). The left Fallopian tube was absent.

The condition found in Case A is probably explained in view of the method of carrying out the operation. We reproduce, through the kindness of Mr J. G. Runciman, M.R.C.V.S., in Fig. 6 the sketch of a uterus which was torn in such a way as, in the hands of an unskilful operator, would have led to the retention of one horn of the uterus together with the corresponding ovary. As the occurrence of imperfect spaying is a matter of no inconsiderable importance from the economic standpoint, it is perhaps pertinent to give an account of the operation, and describe how this accident is liable to take place.

Ovariotomy on the pigling is performed in two positions. In one case the sow is laid flat on her right side with the left hind leg drawn slightly back; in this position the animal is held by an assistant. In the other case the operator, standing upright, swings the young sow by the left leg, puts his right foot on the left side of the neck and slightly arches the body of the sow by bending it across his left leg, thus bringing the left side of the abdomen rather more forward; the surgeon leaning forward to operate. In both cases the drawing back of the left hind leg stretches the flank and abdominal muscles on the left side. The next part of the operation consists in shaving the hair off a small area of skin situated close up to the loin, immediately in front of the thigh. It is in this area that the stab is made through the abdominal wall, admitting the index finger and the withdrawal of the "bed." The stab is made close up to the *loin* about half an inch in front of the ilium. The abdominal wall is pierced by means of a one-edged bistoury in a vertical direction. The blunt edge of the knife is uppermost when the stab is made, or in other words, the back of the blade is against the lumbar muscle. The stab when made by a good operator is only sufficiently extensive to admit of the introduction of one finger into the abdominal cavity. The finger is introduced directly after this incision is made, and the "bed" (*i.e.* the uterus together with the Fallopian tubes) secured by a hooking movement of the finger. Sometimes the middle of one horn of the uterus is all that is at first secured; under delicate manipulation this, in the form of a small contorted, worm-like tube, is generally drawn out through the incision, the rest of the uterus following. It is to be remarked that normally the infantile ovaries remain attached to the horns of the uterus by means of the broad ligament, and so are withdrawn from the body cavity along with them. Occasionally, more especially if the manipulation is not very carefully done, the traction which is needed to draw out the horn of the uterus first seized is so great as to lead to its tearing off, leaving

the rest of the organ behind in the body cavity. This appears to be the way in which imperfectly de-sexed animals are produced in the hands of careless or ignorant operators. Under such circumstances as those described the proper course is to begin again, reintroduce the finger, regrasp the uterus, a matter often of no small difficulty, and extract whatever remains of the organs referred to. The whole internal generative tract beyond the vagina should now be excised. It should be remarked that the division ought to be made externally, *i.e.* the knife is not to be introduced into the body cavity. The tear above referred to should be avoided, for owing to the position of the right uterine horn in the body cavity it is very difficult to reach it with the finger when once it has been severed from the left horn.

Though it so happens in the case illustrated (Fig. 6) that the right horn was the first part removed, Mr Runciman tells us that in his experience the left is usually the one likely to be torn away through this accident. The traction on the organs should be very gentle till the bulk of the uterus is secured; in fact it is safer when only one horn has been secured to draw it between the index and second fingers rather than between the index and the thumb.

Summary of Practical Conclusions.

Black pigment is very frequently, but not invariably present in the mammary tissue of Large-Black, Berkshire and other dark-coloured sows. It is stated that the presence of this pigment (apart altogether from the question of the pigs being "on heat") renders the bacon less saleable, owing to its discoloured or "seedy" appearance[1]. If this is the case, it ought to be possible to remedy this fault, by breeding from individuals in which this pigment is absent, and so building up a strain of increased commercial value.

If on the other hand it be the case that the presence of black pigment is not in itself objectionable, but that the changes in the mammary area due to the occurrence of heat are what are not desired, the remedy is to be sought in spaying, a practice which must be carried out on a sufficient scale and be efficiently performed. That imperfect spaying (in which one, or a part of one, ovary is left behind, while the bed is wholly or partly removed) sometimes happens is shown by the facts described in this paper.

[1] This matter has now (July 9th, 1913) been further investigated and will be treated of again in a future paper.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

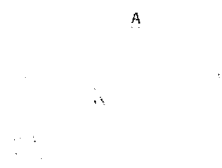

Fig. 6.

The expenses of the investigation described in this paper have been very largely defrayed by a grant made by the Development Commissioners through the Board of Agriculture and Fisheries.

DESCRIPTION OF PLATE.

FIG. 1. Section through developing mammary tissue of foetal pig showing pigment granules in the rete mucosum and in places where this layer is seen dipping inwards to form the glands.

FIG. 2. Transverse section through duct of nipple of Large-Black sow showing pigment in the epithelial cells.

FIG. 3. Longitudinal section through same.

FIG. 4. Left ovary, tube, and horn of uterus found remaining in sow which was supposed to have been spayed (Case A in text). Much reduced.

FIG. 5. Left ovary with large cysts found in sow which was supposed to have been spayed (Case B in text). Reduced.

FIG. 6. The two horns of a uterus which had been accidentally almost completely severed in the operation of spaying (A, the point where the tear had been made. See text).

THE COMPOSITION OF IRRIGATED AND NON-IRRIGATED APPLES.

By J. S. JONES and C. W. COLVER.

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EXPRESSION is frequently given to an apparently widespread belief that irrigated in comparison with non-irrigated fruits are flat in taste and less resistant to the various agencies which effect decay. In support of that belief this statement in substance is almost invariably advanced: "The irrigated fruits contain abnormally high percentages of water and consequently low percentages of solid or dry matter; they are, therefore, deficient in the particular compounds upon which taste and body or solidity of structure depend." We do not presume to say that from analyses alone can the many questions relating to quality in fruits be definitely settled, but since those alleged characteristics of irrigated fruits are charged by this statement to radical deficiencies in certain compounds, it would seem that analytical data would be of material service in the settlement of questions relating to quality. This view of the matter and the fact that here in the north-west, in both the irrigated and the non-irrigated sections, the hardy fruits are grown extensively and shipped to distant markets induced us to undertake some two years ago extensive analyses of fruits grown with and without irrigation. We wish to report here in summarised form the results of that work on the apple; for it, in point of commercial importance, stands pre-eminent among all other fruits grown in the north-west.

It should be particularly noted that none of the samples were secured from especially controlled conditions; all irrigated samples were grown in sections where climate and soil render irrigation imperative, all non-irrigated samples in sections where the annual precipitation varies from 25 to 35 inches and where the soil and

topography of the country render irrigation methods impracticable. The analyses, therefore, indicate the composition of *normal* irrigated and *normal* non-irrigated apples.

For the benefit of those who might wish to compare these analyses with similar ones from other sources, a brief statement will be made in reference to methods of analysis adopted at the beginning of the work and closely adhered to throughout. The determinations were limited to those constituents which are believed to be of greatest influence on taste and other qualities and at the same time subject to widest variation because of environmental or cultural conditions. In all cases the whole fruit exclusive of the stem, seeds, and smallest possible core was used for the analysis.

Total solids were determined by the combined use of the ordinary drying oven and the vacuum desiccator. Portions of the fruit, cut into very small cubes, were kept at a temperature of 40 to 45 degrees Centigrade in the drying oven for approximately 24 hours. They were then removed to the vacuum desiccator where drying was continued to practically constant weight in a vacuum of 20—22 inches and with concentrated H_2SO_4 as the drying agent.

Acids and *sugars* were determined in aliquot portions of the extract obtained by digesting a weighed portion of the finely chopped fruit (twice the normal weight for the Schmidt and Haensch polariscope) for several hours with successive portions of warm water. The acids were titrated with N/10 NaOH and calculated as H_2SO_4. *Invert* and *cane* sugar were determined by precipitation of cuprous oxide from Fehling's solution as modified by Munson and Walker. For the conversion of weights of cuprous oxide to corresponding weights of invert sugar, Munson and Walker's tables were used. *Insoluble solids* were determined by drying at 100 degrees Centigrade to constant weight the residue from the portion digested for acids and sugars.

Nitrogen was determined by the Kjeldahl method on 10—12 gram portions of the fruit; the *crude protein* by multiplying the nitrogen percentage by 6·25.

From raw ash obtained by burning at low red heat in a muffle furnace (leaching and reburning when necessary), *pure ash* was calculated by subtraction of unconsumed carbon, carbon dioxide, sand, and soluble silica.

For the determination of waste, mechanical methods of necessity were resorted to. Although they, in comparison with chemical methods, are incapable of the same degree of accuracy, it should be noted that

TABLE I. *Summary of Analyses of Irrigated and Non-Irrigated Apples.*

Variety and how grown	Number of analyses averaged	Water per cent.	Solids: Total per cent.	Solids: In-soluble per cent.	Sugar: Invert per cent.	Sugar: Cane per cent.	Sugar: Total per cent.	Acids as H_2SO_4 per cent.	Crude protein $N \times 6\frac{1}{4}$ per cent.	Pure ash per cent.	Edible per cent.	Waste: Skins per cent.	Waste: Core per cent.	Waste: Total per cent.
Aiken Red, irr.	3	83·02	16·98	2·76	8·01	2·81	10·82	·233	·175	—	90·99	5·30	3·71	9·01
Arkansas Black, irr.	5	81·59	18·41	3·02	7·58	4·04	11·62	·344	·245	—	91·49	5·76	2·75	8·51
,, ,, non-irr.	6	81·64	18·36	3·64	7·40	4·86	12·26	·306	·361	—	90·02	6·03	3·95	9·98
Ben Davis, irr.	7	83·64	16·36	3·30	6·48	3·58	10·06	·283	·179	—	88·33	6·66	5·01	11·67
,, ,, non-irr.	17	81·31	18·69	4·27	7·58	3·42	11·00	·288	·334	—	85·23	8·45	6·32	14·77
Gano, irr.	3	83·98	16·02	2·92	6·51	3·67	10·18	·316	·150	—	87·27	7·32	5·40	12·73
,, non-irr.	21	82·48	17·52	3·58	7·09	3·72	10·81	·264	·293	·22 *	86·97	7·43	5·60	13·03
Grimes Golden, irr.	1	79·89	20·11	2·70	6·15	6·10	12·25	·322	·194	—	84·95	9·95	5·10	15·05
,, ,, non-irr.	4	82·91	17·09	3·09	5·87	5·29	11·16	·394	·247	—	89·54	5·91	4·55	10·46
Jonathan, irr.	33	82·67	17·33	2·42	7·80	3·30	11·10	·398	·204	·22 *	88·53	6·78	4·69	11·47
,, non-irr.	46	82·61	17·39	2·87	7·80	3·20	11·00	·403	·250	·21 *	87·38	7·03	5·59	12·62
Kinnard, irr.	3	84·02	15·98	2·73	8·72	1·55	10·27	·296	·194	—	90·12	6·03	3·85	9·88
Mammoth Black Twig, irr.	8	82·10	17·90	2·85	8·20	3·54	11·74	·318	·188	—	89·84	6·06	4·10	10·16
Rhode Island Greening, non-irr.	1	82·80	17·20	2·94	6·61	3·44	10·05	·430	·470	—	79·31	—	—	20·69
Rome Beauty, irr.	25	84·81	15·19	2·40	5·88	4·12	10·00	·249	·195	—	90·01	6·04	3·95	9·99
,, ,, non-irr.	33	83·08	16·92	2·98	6·56	4·06	10·62	·308	·266	·22 *	90·00	6·26	3·74	10·00
Spitzenberg, non-irr.	8	82·31	17·69	3·56	6·47	4·03	10·50	·353	·320	—	88·09	7·26	4·65	11·91
Tompkins King, non-irr.	6	81·51	18·49	2·47	6·75	4·98	11·73	·366	·336	—	91·38	5·73	2·89	8·62
Wagener, non-irr.	12	84·98	15·02	2·51	5·46	4·34	9·80	·287	·255	—	90·13	5·98	3·89	9·87
White Pearmain, irr.	3	82·74	17·26	3·10	7·24	3·84	11·08	·222	·206	—	87·42	7·32	5·26	12·58
Winesap, irr.	7	80·63	19·37	2·71	10·40	2·10	12·50	·285	·203	—	88·75	6·59	4·66	11·25
Winter Banana, irr.	6	83·59	16·41	2·70	6·09	3·63	9·72	·386	·248	—	89·48	7·36	3·16	10·52
,, ,, non-irr.	9	83·25	16·75	2·93	6·65	3·61	10·26	·290	·259	—	90·03	6·58	3·39	9·97
York Imperial, irr.	8	83·48	16·52	3·02	7·14	3·79	10·93	·301	·194	—	91·31	5·48	3·21	8·69
Yellow Newtown, irr.	4	82·71	17·29	2·92	7·68	3·72	11·40	·387	·177	—	90·76	5·89	3·35	9·24
,, ,, non-irr.	5	80·48	19·52	4·12	6·96	4·64	11·60	·469	·378	—	—	—	—	—
All Varieties, irr.	116	83·14	16·86	2·66	7·31	3·53	10·84	·322	·200	—	89·40	6·42	4·18	10·60
,, ,, non-irr.	168	82·61	17·39	3·19	7·02	3·82	10·84	·336	·283	—	88·26	6·88	4·81	11·74

* One determination.

the determinations were invariably made by the same analyst. The personal equation was thus eliminated.

In all cases the samples were harvested when the owners of the several orchards in which they grew were harvesting the varieties they represent for market purposes. Moreover all samples were held in storage previous to analysis so as to reach the analyst at approximately the same time they would have otherwise reached the retail trade.

Represented in Table I are the most prominent commercial varieties of Idaho. Possibly other varieties are more prominent in other sections, but these are well known throughout the north-west.

Of a majority of those varieties, eight in all, which were found growing in *both* irrigated and non-irrigated sections, the average irrigated sample contained the smaller percentage of total solids, total sugar, and acid; of all those varieties it contained the smaller percentage of insoluble solids and crude protein. If, in the final summing up, variety distinctions are lost sight of and the classification of samples is made solely on the basis of irrigation or non-irrigation, the average irrigated sample contained the same percentage of total sugar, but smaller percentages of each of the other constituents.

But upon whatever basis comparison of composition is made differences between the irrigated and the non-irrigated in total sugar and acid are so small that we feel justified in the conclusion that there is in the analytical data no substantial basis for the claim that the irrigated apple is inferior in taste. Its uniformly lower content of solids insoluble in water may lend some support to the belief that it is slightly inferior in keeping properties.

When average content of insoluble solids, total sugar, acid, and crude protein were calculated on average content of total solids or dry matter the results shown in Table II were obtained.

Of a majority of those varieties which are represented by both irrigated and non-irrigated samples, the dry matter of the average irrigated sample contained the greater percentage of total sugar; of one-half of those varieties it contained the greater percentage of acid; of all of those varieties it contained the smaller percentage of insoluble solids and crude protein. If variety distinctions are again lost sight of, and, as before, the classification of samples is made solely on the basis of irrigation or non-irrigation, the dry matter from the average irrigated sample contained the greater percentage of total sugar, practically the same percentage of acid, but smaller percentages of insoluble solids and crude protein.

TABLE II. *Insoluble Solids, Sugar, Acid, and Crude Protein calculated on Average Content of Dry Matter.*

Variety and how grown	Number of analyses averaged	Insoluble solids per cent.	Sugar total per cent.	Acids as H_2SO_4 per cent.	Crude protein $N \times 6\frac{1}{4}$ per cent.
Aiken Red, irr.	3	16·24	63·66	1·37	1·03
Arkansas Black, irr.	5	16·41	63·06	1·86	1·33
,, ,, non-irr.	6	19·83	66·78	1·67	1·97
Ben Davis, irr.	7	20·17	61·50	1·73	1·09
,, ,, non-irr.	17	22·85	58·85	1·54	1·78
Gano, irr.	3	18·23	63·55	1·97	·94
,, non-irr.	21	20·43	61·70	1·50	1·67
Grimes Golden, irr.	1	13·43	60·92	1·60	·96
,, ,, non-irr.	4	18·08	65·30	2·30	1·44
Jonathan, irr.	33	13·97	64·05	2·30	1·17
,, non-irr.	46	16·51	63·25	2·32	1·43
Kinnard, irr.	3	17·08	64·26	1·85	1·21
Mammoth Black Twig, irr.	8	15·92	65·59	1·78	1·05
Rhode Island Greening, non-irr.	1	17·09	58·43	2·50	2·73
Rome Beauty, irr.	25	15·80	65·83	1·64	1·28
,, ,, non-irr.	33	17·61	62·77	1·82	1·57
Spitzenberg, non-irr.	8	20·13	59·36	2·00	1·80
Tompkins King, non-irr.	6	13·36	63·44	1·98	1·81
Wagener, non-irr.	12	16·71	65·25	1·91	1·70
White Pearmain, irr.	3	17·96	64·14	1·29	1·19
Winesap, irr.	7	13·99	64·53	1·47	1·05
Winter Banana, irr.	6	16·45	59·23	2·35	1·51
,, ,, non-irr.	9	17·49	61·25	1·73	1·54
Yellow Newtown, irr.	4	16·89	65·93	2·24	1·02
,, ,, non-irr.	5	21·11	59·43	2·40	1·93
York Imperial, irr.	8	18·28	66·16	1·82	1·17
All Varieties, irr.	116	15·78	64·30	1·91	1·18
,, ,, non-irr.	168	18·34	62·33	1·93	1·62

By force of circumstances in the north-west as elsewhere the desiccated apple must be looked upon as an article of growing commercial importance. Differences in composition of dry matter which have just been noted are small; if based on the commercial article they would seem smaller. Therefore as between the desiccated product from the irrigated and that from the non-irrigated orchard there appears to be no substantial basis for market discrimination.

DIGESTIBILITY EXPERIMENTS WITH SHEEP. PARA RUBBER SEED CAKE.

By S. J. M. AULD, D.Sc., Ph.D., F.I.C.,

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THE experimental determination of the digestibility of feeding stuffs has been carried out only to a very limited extent in this country. Nearly all the tables of digestibility-coefficients are of Continental origin, those of Kellner having pre-eminence and being generally adopted. The desirability of carrying out digestibility trials with home-grown feeding stuffs and with new feeding materials obtained from the Colonies and India is, however, apparent, and this is also the case with many of the proprietary feeds which are much used in this country. Tables built up on these lines would materially assist in the calculation of rations, this being at present frequently a matter of difficulty owing to the necessity of estimating an average digestibility, for example, for bye-products incorrectly named or differing slightly from those generally quoted, for materials which may possibly differ considerably in digestibility when grown under different conditions and for the numerous proprietary articles such as the molassès feeds (cf. Goodwin, *Journal Board of Agriculture*, 1911, **18**, 97). A mere extension of the digestibility coefficients already tabulated would be of extreme value, but a judicious replacement or confirmation of the Continental figures for home-grown materials would seem to be still more useful.

Experiments with sheep on these lines were instituted at Wye College and the author intends to continue them at Reading. The trials were carried out with sheep and the results obtained with Para rubber seed cake are offered herewith. The *modus operandi* was essentially that generally adopted and was, in fact, elaborated by Goodwin after personal investigation of Kellner's methods.

As the trials proceeded several modifications were made in the apparatus, particularly with regard to the sheep harness, which is

rather difficult to maintain in position over an extended period. This was largely overcome by making all the connections of broad "elastic," fitted with flat buckles, which allowed any necessary adjustment from time to time. Three sheep (Kents) were used in each investigation, in order to provide sufficient control if any one animal became disordered. Throughout the experiment the boxes were placed in such a manner that each sheep could see every other sheep, since this greatly benefits their docility.

Para Rubber Seed Cake.

This product is the press-cake left after expression of the oil from the kernels of the seed of the Para rubber tree (*Hevea brasiliensis*, Müller Arg.). Enormous quantities of these seeds are available every year and the quantity is certain to increase (cf. *Bull. Imp. Inst.* 1903, 156). Their economic importance would appear to be considerable as they contain 42—49 per cent. of a pale yellow drying oil which has been investigated by Pickles and Hayworth (*Analyst*, 1911, **36**, 493). There is also present a lipoclastic enzyme and a cyanogenetic glucoside (Dunstan, *Proc. Chem. Soc.* 1907, 168). The seeds themselves yield about 0·048 per cent. of prussic acid (Henry and Auld, *Journ. Soc. Chem. Ind.* 1908, **27**, 428). In analogy with linseed cake it appeared therefore, *prima facie*, that the resultant press-cake might be dangerous for use as a cattle food owing to its high content of HCN, which, by calculation, would be expected to be of the order 0·09 per cent., although hot pressing might partially destroy the glucosidoclastic enzyme.

The material used in this investigation was supplied by Professor W. R. Dunstan, F.R.S., Director of the Imperial Institute. It was a light brown very friable cake of rather peculiar, but pleasant smell, and despite the ease with which it crumbled, was found to contain about 20 per cent. of "oil." The cake was examined for prussic acid by the usual methods but none could be detected, either as cyanogenetic glucoside or in the "free" state. This is remarkable and argues a different condition of affairs from those existing in linseed.

Animals given the cake took to it readily and no great difficulty was experienced in this way with the experimental sheep, even when fed over an extended period.

The basal ration adopted in the trials consisted of linseed cake and hay, and the periods adopted were of about one week in duration throughout, viz. (1) seven days basal ration of 300 grams chaffed hay

and 150 grams linseed cake, fed thrice daily, without collecting faeces; (2) the same, faeces being collected; (3) eight days experimental ration (basal ration + 150 grams Para cake); (4) the same, faeces being collected. The sheep No. 3 showed signs of finishing its experimental ration only with difficulty and throughout the fourth period was given an extra 30 grams of a proprietary condimental food at each meal.

The results obtained were as follows:

Analysis of Para Rubber Seed Cake.

Moisture	9·27	per cent.
Crude protein	29·84	,,
Crude fibre	3·15	,,
Ether extract	20·11	,,
Nitrogen-free extractive matter	33·08	,,
*Ash	4·55	,,
* Containing sand	0·23	,,

Analysis of Basal Ration Products.

	Hay	Linseed cake	Condimental food
Moisture	9·70	14·00	11·12
Crude protein	8·97	28·43	11·32
Crude fibre	25·26	11·60	2·64
Ether extract	1·32	7·80	3·36
Nitrogen-free extractive matter ...	48·33	31·15	66·33
*Ash	6·42	7·02	5·23
* Containing sand	1·27	1·18	0·29

The moisture in the hay before grinding was 14·03 per cent. and the analytical figures were calculated back to this basis.

Analysis of Faeces.

	Basal ration			Experimental ration		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
Moisture	12·38	10·33	11·23	9·80	9·89	10·81
Crude protein	9·46	9·02	9·03	10·44	10·45	9·88
Crude fibre	32·40	35·12	32·72	34·00	33·30	30·85
Ether extract	1·09	1·44	1·47	1·49	1·79	1·79
Nitrogen-free extractive matter	32·33	33·86	35·35	34·90	34·70	34·00
*Ash	12·34	10·23	10·20	9·37	10·60	10·67
* Containing sand ...	2·66	2·72	2·38	2·56	2·64	2·55

Sheep No. 1.

Basal ration.—900 grams chaffed hay,
450 grams linseed cake per day.
Average 720 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Hay	126·27	11·25	216·90	76·86	414·09	54·99
Linseed cake	63·00	35·10	52·20	127·92	140·01	31·59
	189·27	46·35	269·10	204·78	554·10	86·58

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Dung	81·90	7·84	232·77	67·74	232·56	74·16
Basal ration digested ...	107·37	38·51	36·33	137·04	321·54	12·42
Coefficient of digestibility		83·07	13·50	66·93	55·05	

Experimental ration.—900 grams chaffed hay,
450 grams linseed cake,
450 grams Para rubber seed cake per day.
Average 680 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Hay	126·27	11·25	216·90	76·86	414·09	54·99
Linseed cake	63·00	35·10	52·20	127·92	140·01	31·59
Para cake	41·71	90·49	14·14	134·28	148·86	20·47
	230·98	136·84	283·24	339·06	702·96	107·05
Dung	66·64	10·13	231·20	70·99	237·32	72·39
Experimental ration digested ...		126·71	52·04	268·07	465·64	
Para cake digested ...		88·20	15·71	131·03	144·14	
Coefficient of digestibility of Para cake		97·4	100*	97·5	96·7	

* Containing sand.

Sheep No. 2.

Basal ration.—Average 630 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Dung	70·11	9·06	221·25	56·70	213·31	64·47
Basal ration digested ...	119·16	37·29	47·85	148·08	340·78	22·11
Coefficient of digestibility		80·45	17·77	72·32	61·45	

Experimental ration.—Average 650 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Dung	64·81	11·71	218·28	68·49	224·17	71·25
Experimental ration digested		125·13	64·96	270·57	478·79	
Para cake digested		87·84	17·11	122·49	138·01	
Coefficient of digestibility of Para cake		97·1	100*	91·2	92·7	

* Containing sand.

Sheep No. 3.

Basal ration.—Average 630 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Dung	70·74	9·25	206·01	56·89	222·70	64·26
Basal ration digested ...		37·10	63·09	147·89	331·40	
Coefficient of digestibility		80·0	23·4	72·2	59·8	

Experimental ration.—Ration + 90 grams condimental food per day.
Average 675 grams dung per day.

	Moisture	Ether extract	Crude fibre	Crude protein	N-free extract	Ash
Ration	230·98	136·84	283·24	339·06	702·96	107·05
Condimental food ...	10·00	3·03	2·37	10·18	59·68	4·71
	240·98	139·87	285·61	349·24	762·64	111·76

Dung	72·96	12·07	202·50	81·67	229·50	78·00
Experimental ration digested		127·80	83·11	267·57	533·14	
Para cake+condiment digested		90·70	20·02	119·69	201·74	
Condiment digested ...		2·58	1·33	7·12	55·15	
Para cake digested ...		88·12	18·69	112·57	146·59	
Coefficient of digestibility of Para cake		97·3	100*	84·0	96·7	
Average coefficient of digestibility of Para rubber seed cake		97·2	100	90·09	95·3	

* Containing sand.

The experiments give results which are fairly concordant, sheep No. 3 showing the greatest divergence from the average. It is noteworthy that this was the animal which received the condimental addition to its ration and this may have had some disturbing influence. In correcting for the amount of the proprietary feed added, the digestibility of its protein content was estimated by treatment with pepsin-hydrochloric acid and was found to be 71 per cent. A microscopical examination showed the food to be composed of about 70 per cent. maize, 10 per cent. crushed beans, about 10 per cent. of desiccated cocoa-nut and the remainder apparently of spices; fenugreek in particular was detected by its smell. It was on this basis that the digestibility coefficients of the proprietary condiment were estimated.

In each case the crude fibre of the Para rubber seed cake shows a digestibility coefficient actually greater than 100 per cent. The reason for this is not very obvious. Possibly the Para cake offered a better medium for bacterial growth, or it may itself contain a cellulose-splitting enzyme. The excess is not, however, very large and there seems little doubt that practically the whole of the small amount of fibre of the rubber cake is digested.

The figures obtained show the Para cake to be one of the most digestible concentrated foods available. This is no doubt partly due to the small amount of crude fibre present. This, and the absence of mucilage as in linseed cake, means a lack of "binding" material and probably accounts for the extreme friability of the product.

The results are offered without prejudice to future work on "digestibility" of food stuffs or to the value ascribed by the author to the present accepted figures.

The author's thanks are due to Mr F. Knowles and Mr T. D. Mosscrop, B.Sc., for their help in carrying out this particular trial and, in particular, to Mr R. H. Carter for his valuable help throughout the whole series of experiments.

A SIMPLE LABORATORY APPARATUS FOR THE CONTINUOUS EVAPORATION OF LARGE VOLUMES OF LIQUID *IN VACUO*.

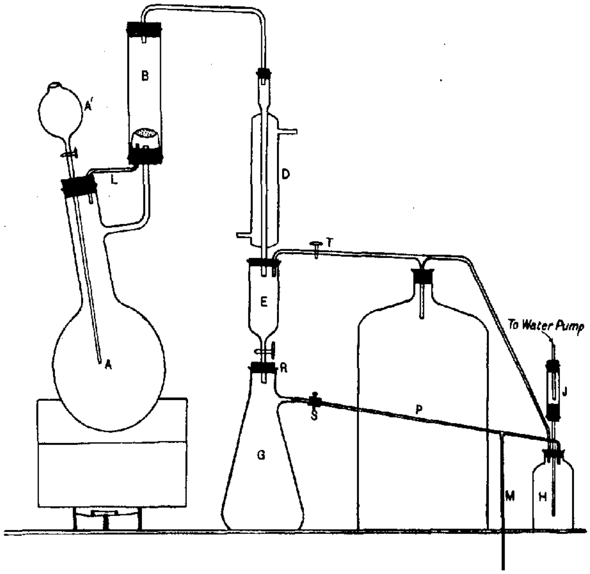
By WILLIAM A. DAVIS.

(*Rothamsted Experimental Station.*)

It is frequently necessary, especially in dealing with plant and animal extracts, to concentrate large volumes of liquid *in vacuo*. In such cases, the operation is often a very tedious one, owing to the necessity of closely watching the apparatus so as to control frothing and avoid the passing over of the liquid into the distillate. Having experienced this, more particularly in the distillation of alcoholic plant extracts, which show a great tendency to froth, the simple apparatus shown in the sketch has been devised which completely overcomes all the difficulties encountered in such work. By means of it large volumes of liquid can be evaporated continuously and the distillate recovered, if necessary in fractions; the apparatus requires practically no watching after the distillation has once been started, and the latter can be left to itself whilst other work is proceeded with. It is only necessary from time to time to renew the liquid in the distilling flask *A*, by means of the dropping funnel *A'*.

The apparatus consists of an ordinary distilling flask with the side-tube bent up and passing into a wide piece of glass tube *B* which serves as a froth-trap; the latter is connected by glass tubing with the condenser *D*, the lower end of which passes through a rubber stopper into the cylindrical dropping funnel *E*, which in turn is connected, as shown, below with the pump-flask *G*, and above with the large reservoir *P*, which serves to take up small variations of pressure and thus ensure a steady vacuum throughout the system.

In this way regular ebullition, without overheating or frothing, is secured.



The vacuum is maintained by means of an ordinary water injector-pump, connected through a Hutchinson regulating valve J (*Chemical News*, 1912, 99) with the bottle H and thence with E and G; a glass cock is interposed at T, whilst S is a screw-clamp which operates on the piece of rubber pressure-tubing connecting G and H. At M a manometer tube is inserted which shows the vacuum throughout the system. The Hutchinson valve takes up large variations in the vacuum due to changes of water pressure, so that by means of this, combined with the regulating reservoir P, changes in the vacuum are reduced to a minimum.

When the liquid in A first begins to boil there is often a great tendency to froth; should this occur, the froth rises into the trap B, breaks against a disc of copper-gauze, and the liquid is returned automatically to the flask through the piece of glass tube L.

The combination E and G allows of the distillate being removed from time to time; whilst the distillation is proceeding, the vacuum

in G is maintained the same as in the rest of the system, so that by opening the glass tap of E the distillate runs down into G. When G is full, and it is required to empty it, the cock on E is closed, and the screw-clamp S screwed down on to the rubber pressure-tube. The latter is then detached from the side tube of G, and the flask G removed from the rubber stopper R, emptied and replaced without interfering with the vacuum throughout the rest of the system. After it has been replaced, S is opened and in a very short time the vacuum is re-established in G, the same as throughout the rest of the apparatus.

It is a very simple matter by introducing T-pieces to run two or more of these distillation apparatus in conjunction with a single vacuum pump and a single regulating vessel P. We have had such an arrangement in continual use now for over a year and it answers all requirements.

All connections must of course be made with rubber stoppers or rubber pressure-tubing.

A STUDY OF THE METHODS OF ESTIMATION OF CARBOHYDRATES, ESPECIALLY IN PLANT-EXTRACTS.

A New Method for the Estimation of Maltose in presence of other Sugars.

By WILLIAM A. DAVIS and ARTHUR JOHN DAISH.

(Rothamsted Experimental Station.)

During an investigation of the carbohydrates present in the mangold leaf, now in progress at the Rothamsted Experimental Station, we have made a special study of the methods of analysis applicable in such cases and have detected certain errors which are likely to occur in this class of work; although the investigation is still incomplete, it seems advisable to describe the methods which we have adopted to obviate these as far as possible.

The study of the accurate estimation of sugars in the complex mixtures occurring in plants may be said to date from the important memoir of Brown and Morris in 1893 entitled "A Contribution to the Chemistry and Physiology of Foliage Leaves[1]"; a few years later Brown, Morris and Millar published their tables[2] of the reducing power of pure dextrose, laevulose, maltose and invert sugar under certain defined conditions with varying concentrations of the sugars and determined the specific rotatory power of pure maltose. Quite recently Parkin[3] in studying the carbohydrates of the snowdrop leaf, which does not contain starch or maltose, tested certain points of analytical procedure necessary in dealing with plant-extracts.

Gravimetric Methods of Estimating Sugars.

In the estimation of mixed sugars such as occur in plant extracts undoubtedly the most satisfactory gravimetric method is to work under

[1] *Trans. Roy. Soc.*, 1893, **63**, 604. [2] *Trans. Roy. Soc.*, 1897, **71**, 72—123.
[3] *Biochem. J.*, 1912, **6**, 1.

the conditions laid down by Brown, Morris and Millar, using the tables already referred to. We have tested the accuracy of these tables by means of carefully purified specimens of dextrose, laevulose, cane sugar and maltose, dried *in vacuo* at 105—106° (except the laevulose which was heated at 75—80° only) under the conditions adopted by Brown, Morris and Millar[1], and have found a satisfactory agreement—that is to within 1 milligram on the weight of copper weighed, which Brown, Morris and Millar regard as the probable degree of accuracy of their method; when 0·20 to 0·35 grm. of copper is weighed the error is therefore within 0·5 per cent.

Possible Error in the Gravimetric Method due to the Action of Fehling Solution on Asbestos.

In our early experiments with purified sugars it was frequently found that the copper oxide weighed in duplicate experiments differed by very large amounts—often not by a mere milligram but by a centigram or more. It was at first thought that this was due to the use of a layer of asbestos in the Soxhlet or Gooch crucible which was insufficiently thick to retain the whole of the cuprous oxide during the filtration, although no oxide was visible in the filtered Fehling solution. On using a much thicker layer of asbestos (½″ to 1″), however, as is usual in sugar-works

[1] Ost (*Chem. Zeit.*, 1897, **21**, 613) in reply to Brown, Morris and Millar's criticism of the value he had assigned to the specific rotatory power of maltose, based on a method in which the hydrated substance was weighed and the rotation for the anhydrous substance derived from this, threw doubt on their values, alleging that, when maltose is heated to a temperature above 95°, even *in vacuo* it begins to decompose, although without showing any external signs of change, the decomposition being indicated only by a falling off of rotatory power. According to Ost the values of the solution-densities and reducing powers given by Brown, Morris and Millar would therefore be only approximately correct ("können principiell nicht als exakt gelten") as slight change had probably occurred in the material used (dextrose, laevulose as well as maltose). This statement is reproduced in von Lippmann's *Chemie der Zuckerarten* (3rd edition, p. 1468), where preference is given to Ost's values of solution-densities.

We have not specially investigated this point but may point out that Ost makes use of a strange argument in support of his case, and refutes himself, when he maintains that the indication of decomposition having occurred in maltose is a *lowering* of the specific rotatory power and yet contends that Brown, Morris and Millar's *high* value for the specific rotation at 15·5° (137·93) as compared with his own (137·46) was due to this cause; had decomposition occurred a *lower* value would be expected. E. Schulze (*Chem. Zeit.*, 1902, **26**, 7) on the other hand maintains that maltose hydrate can be completely dehydrated at 100° in a current of air without any decomposition occurring, and Ling, Eynon and Lane (*7th International Congress App. Chem.*, 1910, **1**, 137) confirm Brown, Morris and Millar's tables of solution-densities. Our own results were also always in full accord with them.

(see Frühling, *Anleitung zur Zuckerindustrie*, 7th edition, 1911, p. 112; von Lippmann in *Chem. Tech. Untersuch.-Methoden*, 5th edition, III. p. 403), it was found that the differences were thereby considerably *increased*. An example will show the character of the results obtained. Using successive portions of 25 c.c. of the same invert sugar solutions (prepared from pure cane sugar) otherwise treated in exactly the same way:

Soxhlet A (previously used for two charges) gave 0·3453 grm. CuO. Soxhlet B (also used for two charges) gave 0·3410 grm. CuO, but Soxhlet C (freshly packed with ¾″ of asbestos and ignited) gave 0·3060 grm. CuO.

In this case the difference between the result C and results A and B is from 0·035 to 0·040 grm.

When thinner layers of asbestos were used in the Soxhlet tube smaller differences were observed, and it was found that by using approximately equal thicknesses of asbestos in different tubes results *differing in duplicate by not more than a milligram* could easily be obtained although these were far from being correct. An example may be given:

Taken 25 c.c. solution = 0·1356 grm. invert sugar.

Thin asbestos layer.

1. CuO = 0·3215 grm = 0·1343 invert sugar = 98·9 %
2. CuO = 0·3211 ,, = 0·1341 ,, ,, = 98·7 ,,
3. CuO = 0·3188 ,, = 0·1330 ,, ,, = 98·0 ,,

Thicker asbestos. 25 c.c. of same solution.

1. CuO = 0·3090 grm = 0·1287 invert sugar = 94·8 %
3. CuO = 0·3100 ,, = 0·1291 ,, ,, = 95·1 ,,

Herein lies probably the principal cause of the not infrequent disagreement between analysts dealing with sugar materials and the doubts which have been expressed as to the accuracy of gravimetric methods. The analyst, using approximately the same thickness of asbestos throughout his experiments, would obtain duplicates in close agreement although the actual result might be considerably at fault.

We became aware of this source of error by observing that, in the case of the particular variety of asbestos we were using (Kahlbaum's specially prepared long-fibre asbestos for Gooch crucibles), the loss of weight experienced with Fehling's solution was particularly pronounced. This asbestos, which when washed with 200 c.c. of boiling water containing 40 c.c. of concentrated nitric acid and subsequently with 300 c.c. of boiling water, showed practically no change in weight (not more than

0·0002 grm.), yet lost considerably on passing through it 50 c.c. of the hot Fehling solution as in an ordinary "Blank" made to determine the correction for self-reduction of the Fehling solution in Brown, Morris and Millar's method. Prior ignition of the asbestos did not alter this property. With thick layers of asbestos ($\frac{1}{2}''$ to $\frac{3}{4}''$) the loss of weight so caused amounted to several centigrams. When successive 50 c.c. quantities of Fehling solution were used, the loss of weight experienced with each successive charge rapidly diminished, and after about the third "Blank" there is the normal *gain* of 0·0015 to 0·0030 grm. CuO, corresponding with the correction necessary to introduce for self-reduction of the Fehling solution (Brown, Morris and Millar, *Trans.* 1897, **71**, 96). It is clear that there is present in the asbestos, as an impurity, some easily decomposable silicate which is gradually dissolved away by the strongly alkaline Fehling solution. The following numbers illustrate this; they were obtained with layers $\frac{1}{2}''$ to $\frac{3}{4}''$ thick of the asbestos.

		Soxhlet 1	Soxhlet 2	Soxhlet 3
1st.	50 c.c. Fehling	−0·0328	−0·0300	−0·0465
2nd.	,, ,,	—	−0·0020	−0·0026
3rd.	,, ,,	—	+0·0021	+0·0016

− indicates loss of weight, + gain of weight.

We find that by digesting the asbestos during 30 minutes with boiling 20 °/₀ sodium hydroxide, and then thoroughly washing with water, an asbestos is obtained which is quite suitable for use in a Gooch crucible or Soxhlet tube as it *undergoes no further perceptible loss when hot Fehling solution is passed through it.* Such asbestos gives the normal increase of 0·0015 to 0·0028 as the correction to be applied for the self-reduction of the Fehling solution, and on passing through it 100 c.c. of boiling 5 °/₀ sodium hydroxide the loss of weight is less than 0·0001 grm.

Different samples of asbestos differ widely in their behaviour with boiling Fehling solution or sodium hydroxide; we have not met with an asbestos which is completely unaffected by these solutions, sample C given below being the best we have as yet obtained. In most cases, the loss is very considerable. In the following examples the asbestos had previously been boiled with hydrochloric and nitric acids, and showed no loss under this treatment. The numbers show the percentage loss of weight on boiling during 30 minutes with 10 °/₀ sodium hydroxide.

A.	White, long fibre	loss 6·94 °/₀
B.	White, long fibre	,, 5·85 ,,
C.	White	,, 0·18 ,,
D.	Blue	,, 0·75 ,,

Although the error that may arise in this way is often very considerable, none of the standard works of analysis we have consulted refers to the necessity of a preliminary treatment of the asbestos with alkali, although von Lippmann (*Chem. Tech. Untersuch.-Methoden*, III. 403) quotes a reference to a paper by Casamajor (*Zeitsch. anal. Chem.*, **22**, 552) in which the ordinary treatment of asbestos with *acids* before use is recommended. In Lippmann's *Chemie der Zuckerarten* (3rd edition, p. 594) the necessity of using asbestos of "guter, reiner und langfaserigen Qualität" is stated and reference made to Maercker (*Oester. Ung. Zeit. für Zuckerind. und Landw.*, **7**, 699; *Zeitsch. Ver. Deutsch. Zuckerind.*, **28**, 797), Killing (*Zeit. angew. Chem.*, 1894, 431) and Elion (*Rec. Trav. Chem.*, 1896, **15**, 116), who had previously pointed out the necessity of using "pure" asbestos. Killing in 1894 went so far as to state that suitable asbestos bids fair to become very scarce or perhaps altogether to disappear from the market and recommended a return to the old method of collecting the cuprous oxide on filter paper.

Weighing the Copper Precipitate from the Fehling Solution.

Numerous papers have been published (cp. Lippmann, *Chemie der Zuckerarten*, 3rd Aufl. 596—598) in which the necessity of reducing the cuprous oxide to metallic copper and weighing as such have been emphasised; the simpler operation of oxidation has been frequently stated to give erroneous results owing to the difficulty of ensuring complete oxidation, the possibility of reduction owing to the action of the flame gases on the cupric oxide, and other causes[1]. Elion for instance (*Zeit. angew. Chem.*, 1890, 325) states that whereas by weighing as copper in sugar estimations he obtained close agreement in four experiments (0·2734, 0·2730, 0·2730, 0·2730), on weighing as cupric oxide he obtained widely discordant results (0·3328, 0·3068, 0·2962, 0·2854).

We have found, on the contrary, that the conversion of cuprous into cupric oxide is practically complete when the following precautions are observed.

1. The precipitate of cuprous oxide is collected in a porcelain Gooch crucible (with sufficient thickness of asbestos), finally washing

[1] In Moissan's *Traité de Chimie Minérale* (Vol. v. p. 459) the oxidation of cuprous oxide on heating in air is said to be incomplete on the authority of Grunhüt (*Chem. Zeit.*, 1894, **18**, 447), Nihoul (*Chem. Zeit.*, 1894, **18**, 881) and Killing (*Zeit. angew. Chem.*, 1894, 431). As we shall show, this is erroneous, unless the blow-pipe flame is used.

with alcohol and ether in the ordinary way, dried at 100°, and after placing in a No. 1 porcelain crucible to act as a container and prevent direct contact of the flame, heated in a fairly powerful flame from a ½" Teclu burner or Fletcher Argand, until the weight is constant; generally, we simply keep the crucible over the flame during half an hour, allow to cool in the desiccator at least one hour, weigh and again heat another 30 minutes. The increase in weight in the second heating seldom exceeds 0·0005 grm.

2. *The blow-pipe should not be used,* as even when the Gooch is shielded by an outer crucible low results are obtained, probably owing to slight dissociation of the cupric oxide at the high temperature (compare Debray and Joannis, *Compt. Rend.*, 1884, **99**, 383 and 688). Its use, too, is more tedious than that of a Teclu burner.

We append a few examples illustrating this. We have never observed the hygroscopic tendency which is sometimes attributed to cupric oxide.

	Wt. Cu_2O	Wt. CuO	Ratio	Remarks
On blowpipe	1. 0·3730	0·4135	1·109	Heated on blow-pipe till constant in weight
	2. 0·3735	0·4140	1·109	
	3. 0·3864	0·4270	1·105	
	4. 0·2587	0·2857	1·105	
On Fletcher or Teclu burner	1. 0·3864	0·4297	1·112	
	2. 0·3767	0·4188	1·112	
	3. 0·3849	0·4271	1·110	
	4. 0·2410	0·2680	1·112	

The theoretical ratio $\frac{2CuO}{Cu_2O} = 1·112$ (Cu = 63·57).

A similar ratio $\frac{CuO}{Cu_2O}$, viz. 1·110 to 1·112, is always obtained when dealing with purified sugar solutions, but when the ordinary solutions obtained from plant extracts (previously treated with basic lead acetate and then deprived of the excess of lead) are used, the ratio is considerably lower (1·095—1·106); whilst it is lowest in estimating the reduction of solutions which have been treated with invertase or yeast, or starch solutions treated with malt extract (see below).

The recommendation is frequently made to weigh the cuprous oxide as such, after drying at 100°, and this method is prescribed for example in the Official and Provisional Methods of Analysis of the Association of Official Agric. Chemists (*U.S. Dept. Agric., Bull.* 107). (Compare Allen's

Commercial Organic Analysis, Vol. I. p. 325.) Whilst this course is safe in the case of solutions of pure sugars, it involves large error when dealing with solutions derived from plant or animal extracts, or when inversion or hydrolysis has been effected by an enzyme, or after fermentation by yeasts, even though alumina cream has been subsequently used to clear the solutions. In such cases the cuprous oxide invariably contains some organic matter, which burns away when it is oxidised, so that the ratio $\frac{CuO}{Cu_2O}$ is thereby diminished[1]; it is probable that, in dealing with yeasts, invertase, etc., the cuprous oxide precipitated contains traces of copper salts of amino-acids as well as the colloidal organic matter carried down by adsorption. In such cases the cupric oxide weighed would be slightly higher than that actually due to reduction only; but the numerous experiments we have made with yeasts, invertase preparations, diastase, etc., would lead us to think that this error is relatively small, and negligible in comparison with other errors of sampling, etc., in this class of work.

We have made it a rule in our work to weigh both the cuprous and cupric oxide precipitate and calculate in each case the ratio $\frac{CuO}{Cu_2O}$. This throws an interesting light on the character of the solution dealt with, and on the purity of the cuprous oxide weighed. *But all calculations for sugars are based only on the weight of cupric oxide actually obtained.* We usually collect many successive charges in the same Gooch crucible one after the other; the same Gooch can be used for 10—20 charges without cleaning, the cuprous oxide from a fresh experiment being collected on top of the previous charge of cupric oxide when the latter is constant in weight.

Description of Heating Bath used.

In order to facilitate the analyses we have made use of the form of water bath shown in the sketch; it consists of a 10″ enamelled iron saucepan, 4½″ deep, into which a false bottom of copper plate is placed, so as to afford a convenient support for the beaker flasks used. The cover of the bath is made of copper and consists of two halves, each

[1] Using invertase in the form of autolysed yeast the ratio of $\frac{CuO}{Cu_2O}$ weighed varies between 1·060 to 1·090 according to the quantity used; with prepared diastase in starch transformations it is slightly higher (1·105—1·106) and with malt extract it again gives low figures similar to those obtained with autolysed yeast.

perforated with two $2\frac{1}{2}''$ holes, the edge of the plate being turned down so as to fit over the bath. Each half of the cover can be lifted off separately so as to admit the beaker flask containing the Fehling solution. We find that 250 c.c. conical beaker flasks, with a top diameter $2\frac{1}{4}''$ and bottom diameter $2\frac{7}{8}''$, give results in close agreement with the Brown, Morris and Millar tables, and are much more convenient for manipulation and heating than ordinary beakers.

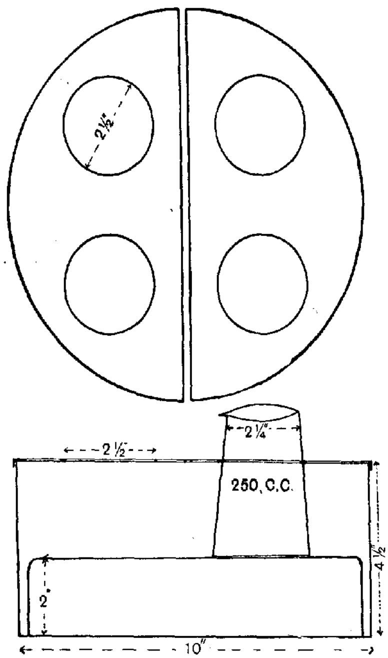


Fig. 1.

Volumetric Methods.

We have spent some time in studying two of the volumetric methods which are generally regarded as the most accurate, viz. that due to Ling and Rendle (*Analyst*, 1905, **30**, 182; compare Ling and Jones, *Analyst*, 1908, **32**, 160), who make use of Fehling solution with ferrous thiocyanate as indicator; and the volumetric permanganate method as carried out by Bertrand (*Bull. Soc. Chim.*, 1906, [iii.], **35**, 1285).

Our sugar solutions were prepared with carefully purified dextrose (recrystallised several times from both methyl and ethyl alcohols) $[\alpha]_D^{15^\circ} = 52{\cdot}6^\circ$, maltose $[\alpha]_D^{15^\circ} = 138{\cdot}0^\circ$ and invert sugar, prepared from cane sugar; in some cases the solutions were made by drying a known weight of the sugar *in vacuo* at 106° (toluene bath) in a small glass flask fitted with a ground-in stopper, and connected with a flask containing phosphoric anhydride, and then dissolving the anhydrous sugar in water and making to a known volume at 15·0°. In others, the concentration was checked by density determinations, using Brown, Morris and Millar's tables[1].

Ling-Rendle-Jones Method.

Dextrose. 1. Taken 0·1975 grm. dextrose per 100 c.c. Found 0·1981 grm.

2. Taken 0·2303 grm. dextrose per 100 c.c. Found 0·2300 grm.

Cane Sugar. (Inverted according to Ling and Rendle.)
Taken 0·2105 grm. per 100 c.c. Found 0·2106 grm.

Maltose. Taken 0·2059 grm. per 100 c.c. Found 0·2056 grm.

These confirm the view generally held that this method is accurate to at least 1 part in 300, or about 0·3 °/ₒ, and we regard it as the most nearly accurate volumetric method at present in use. It is in our opinion preferable to the Bertrand method, both on the ground of accuracy and rapidity.

Bertrand's Method. This method, which consists in dissolving the cuprous oxide in an acid solution of ferric sulphate and titrating the resulting solution with permanganate, had in principle already formed the subject of at least six papers and was provisionally adopted by the U.S. Dept. of Agriculture at least as far back as 1899 (*Bulletin* 46, Bureau of Chemistry), prior to Bertrand's publication of convenient tables, which led to its being widely used in biochemical work.

We have carried out a large number of experiments with this method of which the following are typical: the solutions used were made from carefully purified sugars, and had given good results with the Brown, Morris and Millar gravimetric method and the Ling volumetric method. Bertrand's details were followed precisely.

Dextrose. Taken 0·0658 grm. dextrose.

Found in five experiments average 0·0648 grm., that is an error of

[1] In all cases our numbers refer to true c.c. at 15° C.

about 1·5 per cent. It is noteworthy that Bertrand gives his $[\alpha]_D = 52{\cdot}0°$, whereas the more nearly correct value is probably 52·7°.

Cane sugar. (Invert sugar.)

We obtained, using 0·0961 grm. invert sugar, results which were from 3 to 5 °/$_o$ low. The cause for this probably lies in the fact that the conditions for inverting cane sugar employed by Bertrand, namely heating 4·75 grms. of cane sugar with 50 c.c. of 2 °/$_o$ hydrochloric acid for 10—15 minutes at 100°, invariably leads to the decomposition of laevulose, which is shown by the production of a pronounced yellow colour in the solution; this is visible after 3 to 4 minutes heating. It is not safe to heat cane sugar with 2 °/$_o$ hydrochloric acid above 70°. In inverting cane sugar according to Bertrand's conditions, we invariably observed a decided yellow colour in our solution, whether simply boiled or heated in a boiling-water bath. That decomposition occurs at 100° has been generally recognised since the work of Herzfeld (*Zeit. Ver. Zuck.-Ind.*, 1898, 699 and 742).

Maltose. When working with 0·0824 grm. maltose we obtained results 0·0812 to 0·0826. Here the agreement is better, but the range of probable error is still large—nearly 1 °/$_o$. It is noteworthy that Bertrand made use of hydrated maltose dried in a desiccator over sulphuric acid until constant in weight; to this the objection raised by Brown, Morris and Millar against Ost, that it contains slightly more than the theoretical 5 °/$_o$ of water of crystallisation, for $1H_2O$, can be applied. The $[\alpha]_D$ given by Bertrand, viz. 137·4° (calculated for the anhydrous substance), as compared with what is probably the more correct value, 138·0°, agrees with this view.

In all the above experiments special care was taken to wash the cuprous oxide precipitate very thoroughly with 300—400 c.c. of boiling water, so as to remove the last traces of Fehling solution before adding the acid ferric sulphate solution. In all cases, too, the latter part of the operation was carried out as quickly as possible, so as to avoid the possibility of oxidation which, however, we satisfied ourselves by several experiments with acid solutions of ferrous sulphate is not to be feared under these conditions.

In our hands, the concordance between duplicate experiments made with this method was not such as is desirable in an accurate quantitative process. Bertrand speaks of this method as "un des plus pratiques et des plus précis," an opinion which we cannot endorse; we regard it simply as a fairly rapid approximate method, which may perhaps in certain cases be useful when no high degree of accuracy is required.

In the case of cane sugar Bertrand's tables need revision. On the score of rapidity this method falls far short of the method advocated by Ling, Rendle and Jones.

METHODS OF INVERTING CANE SUGAR IN THE ESTIMATION OF SUGARS IN PLANT EXTRACTS.

In estimating cane sugar in plant extracts it is impossible to invert with hydrochloric acid at 70°, even under Herzfeld conditions as, if maltose is present, a considerable proportion also undergoes hydrolysis to dextrose (see p. 460) and there is also the danger of pentoses undergoing decomposition. It is therefore necessary to make use of invertase or a weak acid, such as citric acid or oxalic acid. We give the particulars of the invertase method later, and will first consider some of the difficulties which may arise in using citric acid.

Boiling 2 °/₀ citric acid[1] has been frequently used for inverting cane sugar and was employed by Campbell (*J. Agric. Sci.*, 1912, **4**, 248) in studying the carbohydrates of the mangold leaf. We can confirm the generally accepted view, that boiling during 10 minutes with 2 °/₀ citric acid completely inverts cane sugar when alone.

1. Taken 1 grm. cane sugar, 4 grms. citric acid, 200 c.c. water, boiled 10 minutes, neutralised to phenolphthalein by sodium hydroxide and made up to 500 c.c.

Solution = 0·2105 grm. invert sugar per 100 c.c.

Found (Ling's method) 0·2089 grm. = 99·3 °/₀ inversion.

2. A duplicate inversion.

Found (Ling's method) 0·2108 grm. invert sugar per 100 c.c. 100·1 °/₀ inversion.

In a series of experiments carried out on mangold leaf extracts, from which tannins, bases, amino-acids, etc. had been removed by basic lead acetate in the usual way, it was found on estimating cane sugar by means of 2 °/₀ citric acid that either cane sugar appeared to be entirely absent, or only a very small proportion seemed to be present. When, however, invertase was used with the same solution, the presence of a relatively large amount of this substance was disclosed. It was ultimately found that the cause of the difference in the two methods was due to the presence in the solution of a large proportion of sodium acetate, which almost entirely inhibits the inverting action of a 2 °/₀ solution of citric acid.

[1] Throughout the percentage of citric acid we give refers to the percentage of the ordinary crystalline acid, $C_6H_8O_7 + H_2O$.

The sodium acetate was produced owing to the necessity of using a very large quantity of basic lead acetate in the removal of the amino-acids, etc., of the leaf; the slight excess of lead was precipitated by sodium carbonate, a relatively large proportion of which however was necessary to neutralise the acetic acid liberated by the amino-acids, tannins, etc. In this way the sugar solutions had become so enriched with sodium acetate as entirely to prevent inversion by citric acid of the concentration used (2 °/$_{\circ}$).

. That this was actually the case is shown by the following experiments.

1. 20 c.c. of a cane sugar solution containing 0·7504 grm. cane sugar was mixed with 50 c.c. of water and 5 c.c. of the ordinary basic lead acetate solution (Allen's *Commercial Organic Analysis*, 4th edition, Vol. I. p. 308) and solid sodium carbonate gradually added so as to precipitate the lead but using as little sodium carbonate in excess as possible; the solution was then diluted to 100 c.c., and 25 c.c. of the filtrate (= 0·1876 cane sugar) neutralised to phenolphthalein by adding a few drops of a citric acid solution. 0·5 grm. of solid citric acid was then added, so as to make a 2 °/$_{\circ}$ solution and the mixture boiled 10 minutes, after which it was cooled, neutralised with sodium hydroxide and heated with Fehling solution under Brown, Morris and Millar's conditions. *No weighable quantity of* Cu_2O *was obtained*, showing that under these conditions no inversion had occurred.

2. *Using Sodium Acetate only.*

It was calculated that 5 c.c. of the basic lead solution would give rise approximately to 1·13 grms. of sodium acetate, $C_2H_3O_2Na, 3H_2O$; 40 c.c. of cane sugar solution (= 1·5008 grms.) and 2·26 grms. sodium acetate was diluted to 200 c.c. (Solution A), and 25 c.c. of this solution (= 0·1876 grm.) boiled during 10 minutes with 0·5 grm. citric acid. The solution was cooled, neutralised, and the reducing power estimated direct under Brown and Morris conditions:

0·1389 CuO = 0·0562 invert sugar = 0·0534 cane sugar
= 28·5 °/$_{\circ}$ inverted.

There had been some inversion, but nearly 75 °/$_{\circ}$ of the cane sugar had been left intact.

Some experiments were made to ascertain the concentration of citric acid necessary to invert cane sugar in presence of considerable quantities of sodium acetate.

It was found that more than 80 °/$_{\circ}$ of the cane sugar is inverted on boiling for 10 minutes with citric acid present to the extent of 2 °/$_{\circ}$ if

normal sulphuric acid is first added until the appearance of the first indication of change of colour with methyl orange.

3. 25 c.c. of Solution A in 2 (= 0·1876 grm. cane sugar) + 1·6 c.c. N-H_2SO_4 (first change of colour with methyl orange) + 0·532 grm. citric acid (= 2 °/ₒ). Boiled 10 minutes, neutralised, and réducing power estimated.

CuO = 0·3973 grm. = 0·1701 invert sugar = 0·1616 cane sugar = 86·2 °/ₒ inverted.

4. Similar experiments were made with the solution obtained in 1 by adding basic lead acetate to the cane sugar solution and subsequently precipitating with sodium carbonate.

25 c.c. (= 0·1876 grm. cane sugar) treated with N-H_2SO_4 (up to first indication of change of colour), solid citric acid added so as to give 2 °/ₒ solution, boiled 10 minutes and neutralised.

(*a*) CuO = 0·3713 grm. = 0·1577 grm. invert sugar = 0·1498 cane sugar = 79·9 °/ₒ inverted.

(*b*) CuO = 0·3910 grm. = 0·1670 grm. invert sugar = 0·1586 cane sugar = 84·6 °/ₒ inverted.

The difference between the two experiments is probably due to a difference in the volume of sulphuric acid added, as the point of change with methyl orange is naturally very indistinct, owing to the sodium acetate present.

5. The same solution as in 4 was used. 25 c.c. (= 0·1876 grm. cane sugar) was neutralised to phenolphthalein by a concentrated citric acid solution, then an equal quantity of citric acid (to neutralise $NaHCO_3$), and finally 1 grm. of solid citric acid added so as to invert with 4 °/ₒ of the latter; boiled 10 minutes and neutralised.

(*a*) CuO = 0·3142 = 0·1309 invert sugar = 0·1243 cane sugar = 66·3 °/ₒ inversion.

(*b*) CuO = 0·3288 = 0·1376 invert sugar = 0·1302 cane sugar = 69·7 °/ₒ inversion.

Here the amount of inversion is less than in 4.

6. *Adding* N-H_2SO_4 *to first change with methyl orange and then citric acid to make exactly* 4 °/ₒ.

25 c.c. of solution 1 (deleaded by sodium carbonate) = 0·1876 grm. cane sugar + 2 c.c. N-H_2SO_4 + 1·08 grms. solid citric acid. Boiled 10 minutes and neutralised with sodium hydroxide.

0·4195 grm. CuO = 0·1811 grm. invert sugar = 0·1720 cane sugar = 91·76 °/ₒ inversion.

7. Same treatment as 6, but citric acid exactly 5 % during inversion.
CuO = 0·4292 = 0·1858 grm. invert sugar = 0·1765 cane sugar = 94·1 %.
Here inversion is still incomplete.

8. By adding sulphuric acid to first change, then citric acid to make a 10 % solution and boiling 10 minutes, inversion is complete.
CuO = 0·4500 = 0·1964 invert sugar = 0·1865 cane sugar = 99·5 %[1].

Inversion with invertase in presence of salts.

It is remarkable that the action of invertase on cane sugar is not interfered with in the least by a proportion of sodium acetate which almost completely prevents inversion by 2 % citric acid.

1. To 25 c.c. of the above solution of cane sugar (= 0·1876 cane sugar) containing 1·13 % of sodium acetate, 1 c.c. of autolysed yeast was added and inverted for 2 hours at 40°. 3 c.c. of alumina cream was added, the solution filtered and the precipitate washed well, evaporating the washings so as finally to have 50 c.c. of the invert sugar solution for reduction under Brown, Morris and Millar conditions.

CuO = 0·4515 = 0·1873 grm. cane sugar inverted = 99·9 %.

2. The same experiment was repeated, but making the cane sugar acid to litmus by adding one drop of *N*-sulphuric acid before adding the autolysed yeast.

CuO = 0·4542 grm. = 0·1885 grm. cane sugar = 100·6 %.

A similar result was obtained by making the solution faintly acid to methyl orange with 1·6 c.c. of *N*-sulphuric acid before inversion; inversion was complete.

Invertase and boiling citric acid do not hydrolyse maltose under the conditions used in dealing with plant extracts.

It was important to make sure that in the inversion of cane sugar by either invertase or boiling citric acid, no maltose was hydrolysed to dextrose.

For this purpose carefully purified maltose was used (see p. 457), four times recrystallised from 80 % alcohol; the solution was standardised by preparing a concentrated solution and ascertaining the density,

[1] No loss of sugar is therefore caused by the use of basic lead acetate, as has sometimes been stated to be the case. This supposed loss has been probably due to incomplete inversion, brought about by the presence of sodium acetate. Parkin has also shown that no loss occurs by a series of special experiments.

using Brown, Morris and Millar's tables, the value so obtained being confirmed by ascertaining the reducing power.

Invertase.

25 c.c. of maltose solution representing 1·0032 grms. anhydrous maltose was digested with 1 c.c. autolysed yeast for 3 hours at 40°; 5 c.c. of alumina cream was added, the solution filtered and washed to 100 c.c. with boiling water. Taken 20 c.c. = 0·2006 grm. maltose.

1. CuO = 0·2700 = 0·1979 grm. maltose = 98·7 %.
2. CuO = 0·2716 = 0·1991 grm. maltose = 99·3 %.
3. CuO = 0·2700 = 0·1979 grm. maltose = 98·7 %.

The slight loss is probably due to retention of maltose by the alumina cream precipitate, in washing which only 70 c.c. of water was used. There has been no increase of reducing power such as would accompany hydrolysis of the maltose to dextrose.

Action of boiling 10 % citric acid on maltose.

A solution of maltose containing 1·941 grms. maltose per 100 c.c. at 15° was used. 10 c.c. of this reduces 0·2642 grm. CuO.

1. 50 c.c. of the maltose solution (= 0·9705 grm. maltose) was boiled 10 minutes with 5 grms. solid citric acid, cooled, neutralised with sodium hydroxide to phenolphthalein and made up to 100 c.c.; taken 20 c.c. = 0·1941 maltose.

CuO = 0·2768 = 0·1951 maltose = 100·6 %.

Very slight hydrolysis of maltose had occurred, representing an increase of reduction of 0·0036 grm. CuO.

2. 50 c.c. of the same solution was made faintly acid to methyl orange, by adding a trace of sulphuric acid, then 5 grms. solid citric acid were added, and the solution boiled 10 minutes and treated as in 1.

Expt. 1. CuO = 0·2677.
Expt. 2. CuO = 0·2715.

Average = 0·2696 = 0·1976 maltose = 101·8 %.

Slight hydrolysis of maltose occurred in these two separate experiments, representing an increase of 0·0035 grm. CuO in one case and 0·0073 in the other.

In another similar case a 1·0032 per cent. solution of maltose boiled with 10 % of citric acid showed in two experiments an average value of 101·6 % maltose. Slight hydrolysis had occurred.

3. Although under the conditions of 1 and 2 maltose showed a slight but distinct hydrolysis, it was found that, under the conditions actually existing in the analysis of plant products, when large quantities of basic lead acetate have to be used, and the excess of lead is removed by sodium carbonate, the sodium acetate formed is sufficient to inhibit all hydrolysis of maltose by boiling 10 °/₀ citric acid; the increase of reduction brought about by citric acid therefore represents true cane sugar. This is, of course, *not* the case if the lead is removed by hydrogen sulphide, because then the solution becomes strongly acid with acetic acid, and unless the acidity is neutralised (to phenolphthalein) by sodium hydroxide prior to the addition of citric acid, an even greater hydrolysis of maltose than is given above would be experienced.

Maltose + sodium acetate and boiling 10 °/₀ citric acid.

(i) 50 c.c. of maltose solution (= 0·5016 grm. maltose), boiled 10 minutes with 5·0 grms. solid citric acid + 0·565 grm. sodium acetate; cooled, neutralised with sodium hydroxide to phenolphthalein and made up to 100 c.c. at 15°.

(*a*) 25 c.c. taken:
(0·1254 maltose) = 0·1724 CuO = 0·1259 maltose = 100·4 °/₀ maltose taken.

(*b*) 40 c.c. taken:
(0·2006 maltose) = 0·2724 CuO = 0·1996 maltose = 99·5 °/₀ maltose taken.

99·95 °/₀

Here no perceptible hydrolysis has occurred.

(ii) The same is true if to the solution of maltose containing sodium acetate, sulphuric acid is added so as to make the solution just change colour with methyl orange.

75 c.c. maltose solution (= 0·7524 grm. maltose) + 0·847 grm. sodium acetate + 4·25 c.c. N-H_2SO_4 + 3·17 grms. citric acid. Boiled 10 minutes, neutralised and made up to 100 c.c.

25 c.c. = 0·1881 grm. maltose taken.

Found 0·2579 CuO = 0·1889 maltose = 100·4 °/₀.

Practically no hydrolysis of maltose by 10 °/₀ citric acid is therefore to be feared. We have however invariably, in our analyses of plant materials, carried out the estimation of cane sugar both by invertase and 10 °/₀ citric acid. This as a general rule has given good agreement and a mutual check is thus obtained on the two methods: this procedure also ensures that the concentration of citric acid has been

sufficiently great to effect complete inversion. In certain cases, when very large quantities of basic lead solution have to be used, it might be necessary to employ citric acid of greater concentration than 10 %, but we have as yet not met with such necessity.

We have found that considerable hydrolysis of maltose occurs on treatment with hydrochloric acid under either the Clerget or Herzfeld conditions usual in the estimation of cane sugar; we have therefore not employed this acid at all in dealing with plant products. (Compare below, p. 458.)

Estimation of Maltose.

It has been frequently proposed to estimate maltose by hydrolysis with dilute hydrochloric or sulphuric acid at 100°, noting the change of cupric reduction or specific rotatory power of the solution after allowing for the inversion of cane sugar present. Under carefully regulated conditions this method gives approximate results in the case of pure maltose or a mixture of maltose and dextrose (cf. Baker and Dick, *Analyst*, 1905, **30**, 79) but it is, as we shall show, inapplicable in all cases when cane sugar and laevulose or pentoses are present, as in the solutions prepared from plant extracts.

Brown and Morris in their classical paper of 1893 used hydrochloric acid under the conditions prescribed by Elion (*Zeit. angew. Chem.*, 1890, 291 and 321), 50 c.c. of the 1 % solution being heated with 3 c.c. of concentrated hydrochloric acid for 3 hours at 100° (boiling-water bath). They observed that the "fall of angle on inversion with acid was, for some unexplained reason, always somewhat less than it ought to be on the supposition that it was due only to the hydrolysis of maltose," and suggested that this "probably indicates the presence of a small quantity of a hydrolysable substance other than maltose and with a less optical activity." In our early analyses of extracts of mangold leaves, we invariably observed the same phenomenon; but as the solutions always contained a brown humus-like precipitate, and had thus undergone considerable decomposition, it appeared that a probable explanation of the increased rotation lay in the destruction of a laevorotatory substance in the solution. The relative instability of laevulose in presence of acids suggested that this was the constituent undergoing change. Experiments with cane sugar and laevulose fully confirmed this view. (Tables I and II.)

TABLE I. *Action of 2·3 °/₀ HCl on Cane Sugar (2 hrs. heating at 100°).*

Conditions. 50 c.c. cane sugar solution + 25 c.c. water + 5 c.c. conc. HCl. After heating, neutralised and made to 100·4 c.c. Taken 20 c.c.

Sugar per 100 c.c. during heating	HCl/100 c.c. during heating	CuO weighed ex. 20 c.c.	Sugar found after heating per 100 c.c.	°/₀ sugar accounted for	Actual weight of sugar destroyed grms.
1·125	2·30	0·4066	0·8321	83·21	0·1679
,,	,,	0·4075	0·8350	83·50	0·1650

TABLE II. *Action of 2·44 °/₀ HCl on Laevulose (2 hrs. at 100°).*

Conditions. 10 c.c. laevulose solution (0·6560 laevulose) + 60 c.c. water + 5 c.c. HCl (36·6 HCl/100 c.c.); after heating 2 hrs. at 100° neutralised with sodium hydroxide and made to 100 c.c. at 15° C.

Conc. of laevulose during heating	HCl/100 c.c. during heating	CuO ex. 25 c.c.	Laevulose found after heating	Laevulose °/₀ destroyed	Actual laevulose destroyed
0·8840 grms. per 100 c.c.	2·44	0·2625	0·4520	31·1	0·2040 grms.
,,	,,	0·2614	0·4500	31·4	0·2060 ,,

In both cases, although the concentrations of acid and sugar are not strictly the same, there has been destruction of, roughly, the same quantity of laevulose; in the case of the pure laevulose the proportion of acid present was somewhat higher, thus accounting for the somewhat greater destruction of the sugar. A considerable quantity of dark brown humus-like substance separated from both solutions, which also became much discoloured, as had been experienced in dealing with the actual plant extracts.

It is clear, therefore, that laevulose is destroyed very largely under the conditions recommended by Brown and Morris for hydrolysing maltose. In the above experiments the heating was only carried out for 2 hours, whereas for the hydrolysis of maltose heating for 3 hours was recommended. We have not made an actual determination of the result of heating during 3 hours with the above concentration of acid, but Tables III and IV show the result of heating for 3 hours with a slightly higher concentration.

TABLE III. *Action of* 4·71 % *HCl on Cane Sugar at* 100° (3 *hours*).

50 c.c. of 2 % cane sugar solution (=1·000 grm.)+15 c.c. water+10 c.c. conc. hydrochloric acid. Heated 3 hrs. in boiling water, neutralised and made to 100 c.c. at 15° C. Used 20 c.c. for reduction.

Conc. of cane sugar during heating, per 100 c.c.	HCl/100 c.c. during heating	CuO from 20 c.c.	Cane sugar accounted for after heating*	% cane sugar destroyed	Actual laevulose destroyed grms.
1·333	4·71	0·2691	0·5310	46·9	0·4690
,,	,,	0·2691	0·5310	46·9	0·4690
,,	,,	0·2713	0·5355	46·45	0·4655
,,	,,	0·2710	0·5350	46·50	0·4650
		Average ...	0·5331	46·65	0·4669

* Using dextrose reducing figure.

TABLE IV. *Action of* 4·58 % *HCl on Laevulose at* 100° (3 *hours*).

10 c.c. of laevulose solution (0·6560 grm. laevulose) +60 c.c. water +10 c.c. conc. hydrochloric acid. Heated 3 hrs. in boiling water, neutralised and made to 100 c.c. Used 25 c.c. for reduction.

Conc. of laevulose during heating	HCl/100 c.c. during heating	CuO from 25 c.c.	Laevulose left	Laevulose % destroyed	Actual laevulose destroyed
0·8288	4·58	0·0339	0·0586	91·07	0·5974 grms.
0·8288	4·58	0·0518	0·0880	86·6	0·5680 ,,
		Made to 101·7 c.c.			

With both cane sugar and laevulose a considerable decomposition was made evident by the production of much brown, humus-like material. Laevulose is thus largely destroyed by heating with dilute hydrochloric acid for such prolonged periods as 2 to 3 hours at 100°. On the other hand, the figures for cane sugar would suggest that the dextrose remains mainly unchanged even after the more prolonged heating with nearly 5 % HCl. This point was specially tested, and Tables V and VI give the results.

TABLE V. *Action of* 2·35 °/$_{\circ}$ *HCl on Dextrose at* 100°.

20 c.c. dextrose solution (0·8216 grm.) + 50 c.c. water + 5 c.c. HCl conc. After heating neutralised and made to 100 c.c. at 15°.

Time of heating	Grms. dextrose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 20 c.c.	Dextrose found	Dextrose °/$_{\circ}$ found	Dextrose actually destroyed
2 hrs. at 100°	1·095	2·35	0·3948			
,,	,,	,,	0·3955			
,,	,,	,,	0·3950			
,,	,,	,,	0·3948			
		Average...	0·3950	0·8140	99·0	0·0076 grms.

Dextrose in 1 °/$_{\circ}$ solution is only slightly decomposed by 2 hours' heating with 2·35 °/$_{\circ}$ HCl at 100°, although even here the dextrose actually destroyed is 7·6 mgrm., but when heated 4 hours in 4·38 °/$_{\circ}$ solution, with a greater concentration of acid (4·15 °/$_{\circ}$) the amount of decomposition is considerable, the actual dextrose destroyed amounting to as much as 0·2400 grm. (Table VI).

TABLE VI. *Action of* 4·15 °/$_{\circ}$ *HCl on Dextrose at* 100°.

75 c.c. of dextrose solution (3·726 grms.) + 10 c.c. conc. HCl. Heated and made to 100 c.c. 20 c.c. diluted to 100 c.c. and 25 c.c. taken for reduction.

Time of heating	Grms. dextrose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 25 c.c.	Dextrose found	Dextrose °/$_{\circ}$ found	Dextrose actually destroyed
4 hrs. at 100°	4·38	4·15	0·4180			
,,	,,	,,	0·4195			
		Average...	0·4188	3·486	93·6	0·2400

It was hoped that it would be possible to arrange the conditions for the hydrolysis of maltose by dilute acid so as at the same time to leave the laevulose intact, but the following experiments showed that it was only possible to obtain anything like complete hydrolysis of maltose under conditions which bring about considerable destruction of laevulose.

TABLE VII. *Hydrolysis of Maltose* by Hydrochloric Acid at 100°.*

20 c.c. maltose solution (=0·8226 grm.) + 5 c.c. conc. HCl + 50 c.c. water. After heating, neutralised and made to 100 c.c.

Time of heating	Grms. maltose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 20 c.c.	Maltose found per 100 c.c.	Maltose % calculated from dextrose formed
1 hour	1·097	2·35	0·4060		
,,	,,	,,	0·4057		
		Average...	0·4058	0·7983	97·05†
2 hours	1·097	2·35	0·4096		
,,	,,	,,	0·4077		
		Average...	0·4087	0·8051	97·88
3 hours	1·097	2·35	0·4080		
,,	,,	,,	0·4091		
		Average...	0·4086	0·8045	97·82

* For the purpose of our experiments Kahlbaum's maltose was recrystallised several times from 80 % alcohol; the commercial material contains generally from 10 to 15 % of dextrin and great care is required in recrystallisation to remove this. We dissolve the maltose in 80 % alcohol, and leave to cool, when the dextrin separates as an oily layer from which the solution of the purer sugar is decanted and the process repeated. Our finally purified material had almost identically the same physical properties and the same reducing power as given by Brown, Morris and Millar (*Trans.*, 1897), within the error of 0·5 %.

25 c.c. of a solution containing 0·8226 grm. anhydrous maltose (dried *in vacuo* at 105°) per 100 c.c. gave

(1) 0·2809 CuO = 0·2059 maltose = 100·1 %
(2) 0·2821 CuO = 0·2067 ,, = 100·5 ,,

† Baker and Dick after 90 minutes' heating found with 1 % maltose + 20 c.c. water + 1 c.c. conc. HCl (sp. gr. 1·16) a hydrolysis of 96·5 %.

The maltose was heated with hydrochloric acid for 1, 2 and 3 hours in a long-necked flask fitted with a reflux and heated in boiling water; before hydrolysis the maltose was approximately 1 % and the acid 2·35 %.

Hydrolysis is not complete after 1 hour's heating at 100°, as is shown by the slight increase in reduction after 2 hours' heating; the increase in hydrolysis is however balanced by the increasing destruction of dextrose, which after 2 hours, according to p. 456, becomes distinctly noticeable.

With slightly stronger acid and longer periods of heating, *lower* results are obtained for maltose, as might be anticipated from the destruction of dextrose which occurs (compare Table VI, p. 456); the following results illustrate this:

TABLE VIII. *Hydrolysis of Maltose by* 4·15 °/$_o$ *HCl at* 100°.

75 c.c. dilute maltose solution (=0·6171 grm.)+10 c.c. conc. HCl; after heating at 100° neutralised and made to 100 c.c.

Time of heating	Grms. maltose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 20 c.c.	Maltose found per 100 c.c.	Maltose °/$_o$ calculated from dextrose formed
3 hours	0·7261	4·15	0·3105		
,,	,,	,,	0·3117		
,,	,,	,,	0·3119		
		Average...	0·3114	0·5905	95·70
4 hours	0·7261	4·15	0·3068		
,,	,,	,,	0·3064		
,,	,,	,,	0·3061		
		Average...	0·3064	0·5805	94·06

Hydrolysis of Maltose at 70°.

Hydrolysis of maltose in 1 °/$_o$ solution by 2·44 °/$_o$ hydrochloric acid at 70°, is very slow, and even after 24 hours' heating only 94 °/$_o$ is converted into dextrose; it is possible that slight decomposition of the dextrose formed may take place during this prolonged period, but this point was not specially pursued, as it was found that laevulose certainly underwent considerable decomposition under these conditions and to an extent rendering it impossible accurately to estimate maltose in presence of cane sugar and laevulose by hydrolysis at this temperature.

The solutions remained colourless throughout the whole time of heating. In calculating the percentage of maltose converted in the above hydrolyses it must be remembered that the reducing power represented by CuO is due to a mixture of dextrose and unconverted maltose. In the last three experiments the original concentration of maltose is slightly different from that of the first three experiments, but the concentration of hydrochloric acid is maintained the same.

TABLE IX. *Action of 2·44 °/₀ Hydrochloric Acid on Maltose at 70°*

20 c.c. of maltose solution (0·7620 grm.) + 50 c.c. water + 5 c.c. conc. HCl; after heating neutralised and made to 100°.

Time of heating	Grms. maltose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 25 c.c.	°/₀ maltose converted
2 hrs. at 70°	1·016	2·44	0·3198	
,,	,,	,,	0·3193	
		Average...	0·3195	28·85
3 hrs. at 70°	1·016	2·44	0·3320	
,,	,,	,,	0·3328	
		Average...	0·3324	34·3
6 hrs. at 70°	1·016	2·44	0·3705	
,,	,,	,,	0·3693	
		Average...	0·3700	52·4

20·69 of another maltose solution (0·7186 grm.) + 49·3 c.c. water + 5 c.c. HCl conc., as above.

Time of heating	Grms. maltose per 100 c.c. during heating	Grms. HCl per 100 c.c. during heating	CuO ex. 25 c.c.	°/₀ maltose converted
12 hrs. at 70°	1·042	2·44	0·4449	
,,	,,	,,	0·4447	
		Average...	0·4448	83·3
18 hrs. at 70°	1·042	2·44	0·4635	
,,	,,	,,	0·4635	
		Average...	0·4635	92·1
24 hrs. at 70°	1·042	2·44	0·4673	
,,	,,	,,	0·4672	
		Average...	0·4673	93·9

TABLE X. *Action of 4·58 °/₀ Hydrochloric Acid on Maltose at 70°.*

20 c.c. of maltose solution (=0·9150 grm. anhydrous maltose) + 50 c.c. water + 10 c.c. conc. HCl was heated 3 and 6 hours at 70°. The solution was neutralised with sodium hydroxide and made to 100 c.c. at 15°. The reducing power was estimated in 20 c.c.

Time of heating	Conc. of maltose during heating grms. per 100 c.c.	HCl per 100 c.c. during heating	CuO ex. 20 c.c.	°/₀ Maltose converted
3 hours	1·144	4·58	0·3955	
,,	,,	,,	0·3916	
		Average...	0·3935	69·9
6 hours	1·144	4·58	0·4300	
,,	,,	,,	0·4300	
		Average...	0·4300	87·6

The curves summarise the above results.

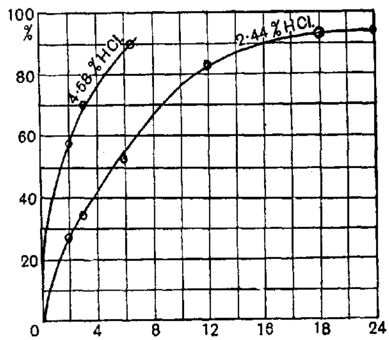


Fig. 2. hours

Action of Hydrochloric Acid on Dextrose at 70°.

The following results show that dextrose is only very slightly changed by prolonged heating (36 hours) with 2·44 hydrochloric acid at 70°; but stronger acid (4·88 °/₀) brings about noticeable decomposition in 24 hours.

TABLE XI. *Action of 2·44 °/₀ HCl on Dextrose at 70°.*

20 c.c. dextrose solution (0·7640 grm. dextrose) + 50 c.c. water + 5 c.c. HCl (36·6 HCl/100 c.c.). Heated 36 hrs. at 70° C.; neutralised with sodium hydroxide and made to 100 c.c. at 15° C. Conc. of dextrose during heating = 1·0187 grm. per 100 c.c.

HCl/100 c.c. during heating	CuO ex. 25 c.c.	Dextrose found after heating	Dextrose °/₀ accounted for	Actual dextrose destroyed
2·44	0·4380	0·7608	99·6	0·0032 grms.

TABLE XII. *Action of 4·88 °/₀ HCl on Dextrose at 70°.*

20 c.c. dextrose solution (0·7640 grm. dextrose) + 45 c.c. water + 10 c.c. HCl (36·6 HCl/100 c.c.). Heated 24 hrs. at 70° C.; neutralised with sodium hydroxide and made to 100 c.c. at 15° C. Conc. of dextrose during heating = 1·0187 grm. per 100 c.c.

HCl/100 c.c. during heating	CuO ex. 25 c.c.	Dextrose found after heating	Dextrose °/₀ accounted for	Actual dextrose destroyed
4·88	0·4341	0·7532	98·6	0·0108 grms.

TABLE XIII. *Action of 2·44 °/₀ Hydrochloric Acid on Laevulose at 70°.*

10 c.c. laevulose solution (0·6560 grm. laevulose) + 60 c.c. water + 5 c.c. conc. HCl. Heated under reflux for 2, 18 and 24 hours at 70°. After heating, neutralised with sodium hydroxide and made to 100 c.c. at 15°. Used 25 c.c. for reduction.

Time of heating	Grms. laevulose per 100 c.c. during heating	Grms. HCl/100 c.c. during heating	CuO ex. 25 c.c.	Laevulose found	Laevulose °/₀ left	Laevulose destroyed grms.
2 hours	0·8840	2·44	0·3687	0·6524	99·50	0·0036
,,	,,	,,	0·3696	0·6540	99·73	0·0020
		Average...	0·3691	0·6532	99·62	0·0028
18 hours	0·8840	2·44	0·3605	0·6368	97·1	0·0192
,,	,,	,,	0·3602	0·6360	97·0	0·0200
		Average...	0·3603	0·6364	97·05	0·0196
24 hours	0·8840	2·44	0·3530	0·6224	94·9	0·0336
,,	,,	,,	0·3504	0·6160	93·9	0·0400
		Average...	0·3517	0·6192	94·5	0·0368

In 0·8840 °/₀ solution laevulose is only very slightly destroyed by 2 hours' heating with 2·44 °/₀ hydrochloric acid at 70 °/₀; but longer

periods of heating bring about considerable destruction, so that it is impossible to hydrolyse maltose even at 70° in presence of laevulose, without destruction of the latter.

TABLE XIV. *Action of 4·88 °/。 Hydrochloric Acid on Laevulose at 70°.*

10 c.c. laevulose solution (0·6560 grm.) + 55 c.c. water + 10 c.c. conc. HCl. Heated 6 hours at 70°. Neutralised and made to 100 c.c. at 15°; taken 25 c.c. for reduction.

Time of heating	Grms. laevulose per 100 c.c. during heating	Grms. HCl per 100 c.c.	CuO ex. 25 c.c.	Laevulose found	Laevulose °/。 left	Actual laevulose destroyed
6 hours	0·8840	4·88	0·3520	0·6200	94·5	0·0360
,,	,,	,,	0·3552	0·6272	95·6	0·0288
		Average...	0·3536	0·6236	95·1	0·0324

In this case the destruction of laevulose in 6 hours is almost as great as with the 2·44 °/。 acid in 24 hours.

Estimation of Maltose in presence of other Sugars by means of Maltase-free yeasts.

It has been known for some years that certain species of yeast do not contain maltase and hence are incapable of fermenting maltose; owing to the impossibility of estimating this substance in presence of the other sugars likely to be present in plant extracts by means of the ordinary methods we decided to ascertain whether these yeasts are suitable for purposes of quantitative estimation of this sugar[1].

For this purpose we fermented a solution of maltose both alone and mixed with cane sugar, with pure cultures of *S. exiguus*, *S. anomalus* and *S. marxianus*, which Dr H. B. Hutchinson was good enough to prepare for us. To the solution of sugar, 5 c.c. of yeast water was added and the mixture, after being sterilised by 10 minutes' heating in the autoclave, was inoculated with a trace of the pure yeast from an agar-yeast-water tube-culture.

[1] Baker and Dick (*Analyst*, 1905, **30**, 79) have suggested the use of *S. marxianus* for *detecting* maltose in presence of dextrose, by the increase of specific rotation and drop in reducing power which occur in fermenting the mixed sugars with this yeast; they fermented, however, only for a relatively short time and did not completely remove the dextrose as we have done, so as to make the method a quantitative one.

The flask containing the liquid is stoppered with cotton wool in the usual manner and incubated at 25° for three to four weeks. When the fermentation is complete, 5 c.c. of alumina cream is added and the solution boiled to expel alcohol: it is then filtered, and the precipitate washed until the filtrate has a volume of 100 c.c.

An aliquot portion (50 c.c.) can then be used for the cupric reduction.

TABLE XV.

	Yeast	Time	CuO from 50 c.c.	Maltose found	% Maltose found
1st Series					
Maltose (0·2006) + Cane Sugar (0·3751)	*S. exiguus*	17 days	0·1525	0·2224	110·8
	S. anomalus	23 ,,	0·1355	0·1974	98·4
	S. marxianus	21 ,,	0·1348	0·1962	97·4
Maltose only	*S. anomalus*	21 ,,	0·1360	0·1980	98·7
	S. marxianus	21 ,,	0·1340	0·1952	97·3
2nd Series					
Maltose (0·3704) + Cane Sugar (0·2000)	*S. exiguus*	31 ,,	0·2535	0·3712	100·2
	S. marxianus	31 ,,	0·2548	0·3736	100·8
	S. marxianus	31 ,,	0·2547	0·3734	100·7

In the first series the high result with *S. exiguus* is undoubtedly due to incomplete fermentation, the time being insufficient. The slightly low results in the other experiments in this series are due either to the maltose used being slightly contaminated with dextrose, or, more probably, to experimental error on the relatively small quantity of maltose taken. In the second series a very carefully purified maltose was used and a larger quantity taken so as to minimise the proportional error. In this case it will be seen that in spite of a very vigorous growth of yeast the maltose is quantitatively recovered, whilst the cane sugar is completely fermented away.

By the use of these special maltase-free yeasts it is therefore possible accurately to estimate maltose in presence of other sugars (dextrose, laevulose and cane sugar) which are completely fermented by them. We have applied this method now for some considerable time to the analysis of plant extracts and find that it is generally necessary also to introduce a correction for the presence of reducing

substances such as pentoses[1] which remain after fermentation by the yeast is complete. This correction is obtained by carrying out fermentation with a pure culture of ordinary distillery or baker's yeast, which ferments away the maltose as well as the other sugars, but leaves a slight residual reduction due to pentoses, etc.; on subtracting this value from the reducing value obtained by using the maltase-free yeasts, the cupric reduction due to maltose alone is obtained.

Estimation of Maltose in Plant-extracts.

For this purpose the plant extract, from which amino-acids, tannins, etc., have been removed by means of basic lead acetate, has to be entirely freed from lead before the yeasts will grow satisfactorily. For this purpose two methods may be used:

1. Solid sodium carbonate is added little by little until no further precipitate is produced. The filtrate, which still contains traces of lead, is made slightly acid with hydrochloric acid and treated with hydrogen sulphide as in 2.

2. The excess of lead is removed directly by hydrogen sulphide. In this case the solution becomes strongly acid owing to the presence of free acetic acid and must be partly neutralised by adding dilute sodium carbonate solution until the reaction is faintly but distinctly acid to litmus paper (see p. 467).

It has been our custom to carry out five fermentations with each plant extract to be analysed, viz. one each with *S. anomalus*, *S. exiguus* and *S. marxianus*, and two with distillery yeast. The agreement between the results with the different special yeasts has generally been entirely satisfactory. Certain differences however in the behaviour of the yeasts may here be noted.

S. anomalus grows rapidly and gives a very bulky mass of yeast; it is apparently less efficient as a sugar remover than *S. marxianus* or *S. exiguus*, that is, it is slower in its action, and a greater yeast growth accompanies the removal of a certain weight of sugar. It shows a decided tendency to grow at the surface as a film. The cuprous oxide obtained in the subsequent reductions often filters very slowly.

S. marxianus is more sensitive to slight excess of acid than the

[1] The pentoses present in plant extracts are apparently not fermented by either baker's yeast or the special maltase-free yeasts we have used. Experiments on this point are still in progress.

other two yeasts, and refuses to grow in acid solutions in which the others readily multiply. *S. exiguus* is probably the most convenient for general use.

Pentoses.

Pentoses are generally present to some considerable extent in the solutions obtained by the extraction of foliage leaves, and, after the treatment with basic lead acetate and subsequent removal of excess of lead, exercise a reducing action on Fehling solution. In view of recent work, especially that of Levene and Jacobs (*Ber.*, 1909, **42**, 2469, 2474, 2703; *Biochem. Zeit.*, 1910, **28**, 127), it is probable that pentoses play an extremely important part in the leaf's activity, especially *d*-ribose, which is an essential constituent of the nucleus of both plant and animal cells; the pentoses present in the aqueous alcoholic extract of leaf tissue are very possibly largely derived from nucleic acids. There is, however, also the possibility of the presence of arabinose and xylose as well as methylpentoses. In calculating the proportions of dextrose and laevulose we therefore have to make allowance, after subtracting the reduction due to the maltose (when this is actually present), for the pentoses; here we are faced with the difficulty that we do not know in any particular case what pentoses actually are present or what is their reducing power under the special conditions of the actual analysis.

In the present state of our knowledge we must be content with approximations, but the nature of these will affect the accuracy of the values for dextrose and laevulose, which are calculated from the primary reducing power of the plant solutions.

We have in our experiments estimated the total pentoses by distilling a quantity of the solution of which the "direct" reducing power of the sugars is determined, with hydrochloric acid according to the ordinary A.O.A.C. method (see Allen's *Commercial Organic Analysis*, I. 401; Bulletin 107, U.S.A. Dept. of Agriculture), weighing the furfural formed as phloroglucide. We intend to introduce a correction for the pentoses by ascertaining their reducing power under the conditions in which we have made our analyses.

Analysis of Plant Extracts.

The scheme of the analysis of a plant extract, such as that of foliage leaves, which we have adopted, may be outlined as follows:

Extract evaporated *in vacuo* (700 to 740 mm.) to small volume. Made to a definite volume, say 500 c.c.

2 portions of 20 c.c. each evaporated to dryness and dried *in vacuo*, 18 hours at 100°. This gives *total dry matter* in extract. (20 c.c. kept as reserve in case of accident.)	440 c.c. treated with basic lead acetate, filtered under pressure on Büchner funnel and washed to known volume (2 litres) = Solution A.

300 c.c. of Solution A is deleaded by solid Na_2CO_3 and made up to 500 c.c. = Solution B.

1. 25 c.c. of B is used for *direct reduction* and *polarised*[1]; the reduction is due to *dextrose, laevulose, maltose, pentoses.*

2. *For cane sugar.* 50 c.c. of B is inverted:

(*a*) *By invertase.* Make faintly acid to *methyl orange* by a few drops of concentrated sulphuric acid, and add 1—2 c.c. autolysed yeast and two or three drops of toluene and leave 24 hours at 38—40°C. After this period, add 5 to 10 c.c. alumina cream, filter and wash to 100 c.c. Take the reducing power of 50 c.c. (= 25 c.c. B) and polarise.

(*b*) *By* 10 °/。 *citric acid.* Make faintly acid to methyl orange by a few drops of conc. sulphuric acid and add a weighed quantity of citric acid crystals so as to have 10 °/。 of the crystalline acid ($C_6H_8O_7 + H_2O$) present. Boil 10 minutes, cool, neutralise (to phenolphthalein) with sodium hydroxide, make to 100 c.c. and determine *reducing power* of 50 c.c. (= 25 c.c. B). Polarise.

Cane sugar is calculated from the increase of reducing power or change of rotation caused by inversion. The values obtained by the two methods *a* and *b* should agree closely.

3. *For maltose.* Another 300 c.c. of Solution A is deleaded by means of hydrogen sulphide and filtered, the precipitated sulphide being washed until the total volume of filtrate and washings is about

[1] The polarisation of these dilute solutions is usually small and it is therefore necessary to take the reading with a long tube (at least 200 mm. in length), with an instrument reading accurately to $\frac{1}{100}$°, the temperature being maintained constant at 20° C. within $\frac{1}{10}$°. It is an easy matter, using a Lowry thermo-regulator, and circulating the water by means of a small pump, to keep the temperature constant to $\frac{1}{100}$°; but we find that differences of temperature less than $\frac{1}{10}$° hardly make a perceptible difference in the readings with such dilute solutions as we have worked with.

450 c.c. Air is then sucked through this for about $1\frac{1}{2}$ hours to expel hydrogen sulphide, a very little ferric hydroxide is added to remove the last traces of the latter, and the solution is made to 500 c.c. It is filtered and

50 c.c.	fermented	(*a*)	with	*S. marxianus*
"	"	(*b*)	"	*S. anomalus*
"	"	(*c*)	"	*S. exiguus*

and two lots *d* and *e* of 50 c.c. are fermented with baker's yeast. It is generally necessary, in order to ensure good growth of the yeast, to reduce the acidity by adding 2 to 5 c.c. of *N*-sodium carbonate to the 50 c.c. to be fermented; 5 c.c. of sterilised yeast water is also added, the mixture is sterilised in the usual way and inoculated in the inoculating chamber with the pure culture of yeast. It is then stoppered with cotton wool and the yeast allowed to incubate for 21 to 28 days at 25°.

After completion of fermentation, 5 c.c. alumina cream is added, the solution made to 100 c.c. at 15°, filtered and 50 c.c. used for reduction. The *difference* between the *average* reduction with *a*, *b*, *c* and the average of *d* and *e* gives the reduction due to *maltose*.

4. *Pentoses.* These are approximately determined in 50 c.c. of A by distilling with hydrochloric acid according to the A.O.A.C. method, weighing as phloroglucide.

5. When the reduction in 1 due to pentose and maltose has been allowed for, the remaining direct reduction is due to dextrose and laevulose; the actual proportions of these two sugars are calculated from the reducing power combined with the corrected specific rotation as suggested by Brown and Morris in their 1893 paper.

In conclusion, we wish to express our best thanks to Dr H. B. Hutchinson for his kindness in preparing pure cultures of the yeasts employed and assistance in their manipulation and to Mr G. C. Sawyer for help in the analyses.

Summary.

1. Certain sources of error encountered in the estimation of sugars in plant extracts are dealt with. Large errors in the gravimetric method may be obtained unless special care is taken in purifying the asbestos by boiling for at least 30 minutes with 20 °/₀ sodium hydroxide. Weighing the reduced copper as cuprous oxide is likely to give rise to

large error, and a process of weighing as cupric oxide, with certain precautions, is recommended.

2. The volumetric methods of Ling and of Bertrand have been studied; the former is preferable in all respects to the latter, which we regard as only roughly approximate.

3. In dealing with plant extracts, owing to the accumulation of sodium acetate in the solutions analysed, inversion by citric acid of lower concentration than 10 °/₀ is generally incomplete. Inversion by invertase is, however, not interfered with by this salt. To estimate cane sugar inversion both by invertase and 10 °/₀ citric acid is recommended. No loss of sugars occurs owing to the use of basic lead acetate as has been sometimes stated; the supposed loss is probably due to incomplete inversion caused by the presence of sodium acetate.

4. It is shown by a detailed study of the action of dilute hydrochloric acid on different sugars that it is impossible completely to hydrolyse maltose at either 70° or 100° without simultaneously destroying large quantities of laevulose or dextrose.

5. The only available method for the accurate estimation of maltose consists in the employment of special maltase-free yeasts, such as *S. exiguus*, *S. marxianus* or *S. anomalus*, introducing a correction (for pentoses, etc.) obtained by a special fermentation with baker's or brewer's yeast.

6. A scheme for the quantitative estimation of sugars in plant material is given.

THE MOVEMENTS OF SOIL-WATER IN AN EGYPTIAN COTTON-FIELD.

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THE attention of all scientists in Egypt has been more and more closely directed to the problems of Soil-water since about the year 1907. In the first instance this attention was of necessity devoted to the water-logged layer of the soil and sub-soil, to the fluctuations of this layer in time and in space, and to its effects on the cotton plant.

The main outlines of this problem having been clearly distinguished, and due recognition made of the important fact that the growth of cotton is more usually "limited" by the soil-water than by any other factor, except during the first half of development, it was natural that attention should then be turned to the effects of variations in the water-content of soils above the saturated layer, and to the physical problems involved, with the object of obtaining knowledge which could be translated into irrigation practice.

Work on these latter lines has been commenced by other workers, but since the problem lies, in part at least, within the domain of the plant physiologist, the writer has attacked it independently with the object of tracing a base-line from which less minute observations might be worked out. The results completely confirm the general statement of the case from the botanical view-point which the writer has made elsewhere[1], and also provide some data in respect of soil-physics.

EXPERIMENTAL METHODS.

Site and area. A random choice was made of an area ten metres by twenty in a field surrounding the Botanical Laboratory at Giza. This area was surrounded by similar land in all directions. The land

[1] Balls, W. Lawrence, *The Cotton Plant in Egypt*, London, 1912.

was sown in Assili cotton, planted at the usual spacing of the district, namely, 45 cm. apart on ridges at 75 cm., with two plants left in each hole. Cultivation was conventional, and an average yield was obtained from the land, to wit, about 500 pounds of lint per acre.

The small size of the area actually sampled was almost inevitable, on account of the great diversity of sub-soil which may be encountered in adjacent borings. This applies to any alluvial soil, and particularly to the land around our laboratory, where the junction between a bed of loam and a bed of clay has been found to slope half a metre in ten metres. By repeated sampling of the same area on a systematic scatter, it was possible to eliminate these diversities almost completely. A plan was kept which showed the exact position of each bore.

Within this area of 200 sq. m. the geological structure of the soil was almost constant, in the following way:

Surface to 30 cm.[1]	...	...	Made soil.
30 cm. to 90 cm.	...	...	Stiff clayey soil.
90 cm. to 200 cm.	...	...	Loam to sandy loam.
Below 200 cm.	...	...	Stiff clayey soil.

The clayey soils are not true clays, but are of similar physical properties, owing to the fineness of their particles. When their water-content is reduced to 10 °/₀ they are almost impenetrable with a Fraenckel borer, and the only interruption of the series here described was due to a breakage of the boring tool from this cause.

Sampling. The samples were taken at every twenty centimetres, from 20 cm. to 160 cm., one bore of eight samples being taken on each date. Two bores were made each week from May 4th to September 28th, or five months in all.

The mean dry-weight of the samples was six grams. On opening the chamber of the borer they were rapidly transferred to the nickel-plated brass tubes with screw caps, adopted from the ordinary shaving-soap tube by my colleague, Mr F. Hughes. These tubes have been completely satisfactory. Care was taken to screen the tubes from the sun, both before, after, and during the filling, and they were weighed immediately after the bore had been finished. Drops of perspiration provided another error to avoid.

The drying of the samples was done in the tubes, and standardised as follows. The caps of the tubes having been removed, the latter

[1] To avoid repetition, all measurements downwards from the soil surface are simply stated in centimetres without adding "below soil surface."

were arranged in a circle inside the water-oven at 100° C. A glass pipe descended through the roof of the oven to a level with the tops of the tubes, and equidistant from all of them; this pipe led to the vacuum water-pump, which was kept running continuously during the six hours which drying lasted, and so provided a gentle draught over all the tubes equally, removing the evaporated water.

All determinations are thus fairly comparable, even though the absolute values may not have been obtained. Samples from the loamy layer would dry more rapidly than those from the clayey layer, but the error is not serious, since a sample from any one depth was usually of the same texture at all times. This uniformity is shown on inspection of the deep-soil records for the early summer (Fig. 1).

Fresh sample less dry sample gave water-loss. Dry sample less tare gave dry-weight. The water-content was then expressed as the percentage of water-loss to dry-weight, as in Table I.

All bore-holes were filled up before a watering, to avoid abnormal vertical distribution of water.

THE WATER-TABLE.

It will be seen that in the last few bores of the list in Table I the water-content of the soil has risen abnormally, and that traces of an increased water-content appear even before this abnormal rise (Fig. 2*d* and 2*e*). This is due to the rise of the water-table, controlled by infiltration from the Nile itself, about a mile away. In the bore-holes of the 21st and 25th of September, the water had risen on the following days to 107 and 105 cm. respectively. This level is higher than that indicated by the soil analyses, which were plainly not completely saturated until below 120 cm. The discrepancy may be due to capillarity, to a slight artesian effect, or to compression of the soil air between the water-table and the clay above, which would be released by the boring. The effect is not one which concerns us at present, but it should be noted as a discrepancy.

SATURATION CONTENT.

Mention of the saturation by the water-table involves reference to the amount of water required to saturate the various layers sampled. In default of precise determinations of this, sufficient indication should be given by the run of the curves themselves. The saturation value is not less than 35 per cent. in the loam.

Date	Depth in cm. 20	40	60	80	100	120	140	160	Number of bore on Plan	Notes
May 4	24·8	24·2	28·5	32·9	24·9	32·7	30·9	30·8	1	
8	22·0	24·2	27·7	32·7	24·3	29·4	30·7	30·8	2	
11	9·1	19·3	21·1	28·4	27·0	33·2	28·9	29·5	3	
15	8·0	16·6	23·4	27·4	23·3	30·8	30·3	29·8	4	
18	5·0	18·3	26·9	30·2	22·3	27·0	30·9	31·8	5	
22	6·2	18·3	27·2	32·0	24·9	30·3	32·5	34·6	6	
25	10·5	25·1	25·3	25·6	24·4	28·3	24·3 *	27·2	7	* Hard clay, not sand.
29	6·4	17·4	24·0	30·7	(31·3 ?)	29·2	31·3	28·9	8	
(Irrigated May 30) June 2	26·4	25·6	29·5	29·9	24·3	28·0	33·1	33·6	9	
5	19·0	23·7	30·1	30·9	27·0	33·3	29·4	29·7	10	
8	22·8	23·2	27·8	29·7	29·3	31·8	30·9	30·3	11	1, 2, 5, 6 Clay; 3, 4, 7, 8 Loam.
12	16·2	20·0	25·7	29·9	28·0	33·4	30·6	31·4	12	
16	7·1	10·6	24·8	29·8	22·5	32·2	32·5	31·7	13	
19	7·3	13·4	25·4	30·4	21·0	30·7	28·9	30·1	14	
23	(17·1 ?	14·1 ?	27·0 ?	11·5 ?)	17·3	24·8	32·5	32·0	15	Surface layers very hard.
(Irrigated June 23) 26	28·1	26·2	29·7	29·7	28·0	26·9	33·6	35·4	16	
29	25·4	25·1	25·9	29·2	25·9	29·3	32·1	31·9	17	Algae on surface.
July 3	22·5	22·7	25·6	29·1	28·1	32·0	29·5	30·7	18	
6	20·4	20·4	26·9	30·9	18·9	30·7	31·1	33·3	19	1–4 Clay; 5 S. Loam; 6 C. Loam; 7, 8 Loam.
10	19·0	18·1	20·0	29·4	22·6	24·7	33·1	31·7	20	5–8 Sandy.
13	16·7	17·2	12·6	15·7	28·6	28·3	32·0	34·8	21	1, 2, 5 Clay; 3–4 S. Clay; 6, 7 Clay Loam; 8 Loam.
17	13·1	14·1	13·9	13·3	24·2	29·8	30·9	34·3	22	
(Irrigated July 17) 20	29·6	25·4	26·6	30·8	26·5	32·1	30·3	30·1	23	
24	25·2	22·4	19·4	25·1	31·8	28·6	33·1	28·0	24	
27	22·8	22·3	18·0	16·8	25·0	30·1	32·1	34·2	25	
31	23·0	22·4	23·7	27·4	18·9	32·1	29·1	30·1	26	
Aug. 3	17·2	15·6	14·0	12·6	24·6	22·3	30·2	30·6	27	
7	17·2	15·1	17·4	20·6	10·8	16·0	30·8	29·2	28	
10	—	—	—	—	—	—	—	—	29	Borer broke at 50 cm.
(Irrigated Aug. 13) 14	30·7	26·1	23·2	24·8	29·3	29·0	30·4	30·9	30	Twenty hours after irrigation.
17	27·3	22·7	20·2	18·9	23·8	25·2	30·6	28·9	31	
21	25·2	22·1	*	15·8	10·5	24·6	30·8	32·3	32	* Sample spilt in drying.
24	24·5	22·5	26·3	29·9	24·6	28·4	30·3	30·0	33	
28	21·0	15·8	14·6	18·9	32·2	28·4	32·5	32·8	34	
31	22·6	16·1	17·6	24·8	30·3	31·4	31·6	30·1	35	
Sept. 4	19·2	21·0	26·2	30·2	23·3	32·3	30·1	33·4	36	
7	23·6	24·3	26·9	31·2	28·3	28·5	33·0	30·9	37	
11	19·7	22·9	24·2	29·8	27·3	30·9	34·8	34·9	38	
14	27·7	26·6	25·6	31·4	34·8	34·1	34·2	37·0	39	
18	25·8	28·0	28·0	31·2	30·0	31·6	34·3	32·3	40	
21	27·1	26·1	28·3	32·1	35·4	29·2	35·5	33·2	41	Sampling continued once a week only *till* November.

Distribution of water in the soil of a cotton-field.

May—October, 1912. Giza Botanical Laboratory.

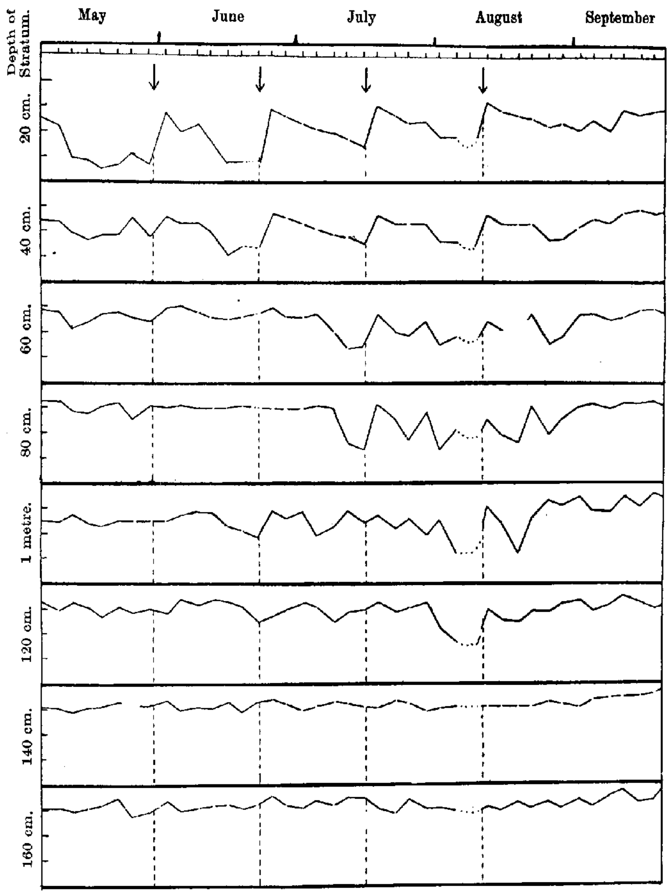


Fig. 1.

Ordinates represent percentage of water. Guide-scales on left showing 10 %, 20 % and 30 %.
Arrows denote dates of irrigation.
Plotted from Table I directly.

THE DRYING EFFECT OF THE ROOT.

On plotting the curves of water-content from the table already given we obtain Fig. 1.

The most striking feature of this figure is the progressive drying of the deeper and deeper layers as the root reaches further and further down, causing a reversal of the Humidity Gradient (Fig. 2*a* and 2*e*). The writer has discussed the general bearing of this on root-function elsewhere[1], though with much more slender evidence than these curves provide.

The proximity of the water-table to the surface throughout the season is a distinct disadvantage of these records. From May till mid-August it remained fairly steady at a depth of two metres, *i.e.* within 30—40 cm. of the lowest sample taken, or sometimes nearer, *e.g.* July 13th and 17th. The wetting of the lower layers of soil due to this proximity (Fig. 2*d*) serves to obliterate the root-drying effect, which had formerly been recorded at greater depths[1]. On the other hand, we have indirect evidence that the plant draws on the water-table, or rather on the water absorbed by capillarity into the soil immediately above the water-table, for a portion of its water supply.

The same conclusion follows when we consider that the water-table falls during the summer in sites where under-drainage can scarcely take place. In other words, more water may be evaporated from the plant and soil together than is applied in irrigation.

A computation of the total loss of water from the crop during the period covered by the curves, based on the changes in water-content, leads to the same result.

TOTAL TRANSPIRATION OF THE CROP.

The following table shows the method by which an attempt to estimate this figure has been made.

[1] *Loc. cit.*

TABLE II. *Distribution of Water in Soil.*

Taking means of three borings to eliminate the known soil-diversity at 100 and 120 cm.

A. The week immediately after irrigation.
B. The week immediately before irrigation.

Period	Dates		Depth in cm. 20	40	60	80	100	120	140	160
a	*A*.	May 4-11	18·6	22·6	25·8	31·3	31·4	31·4	30·4	30·4
	B.	,, 22-29	7·7	20·3	25·5	29·4	26·9	29·3	29·4	30·2
		Loss	**10·9**	**2·3**	**0·3**	**1·9**	**4·5**	**2·1**	**1·0**	**0·2**
b	*A*.	June 2-8	22·7	24·2	29·1	30·2	26·9	31·0	31·1	31·2
	B.	,, 16-22	7·2	12·0	25·1	30·1	20·3?	29·2?	31·3?	31·2?
		Loss	**15·5**	**12·2**	**4·0**	**0·1**	**6·6?**	**1·8?**	**0·0?**	**0·0?**
c	*A*.	June 26	25·3	24·6	27·1	29·3	27·3	29·1	31·7	32·7
	B.	July 10-17	16·3	16·5	15·5	19·5	25·1	27·6	32·0	33·6
		Loss	**9·0**	**8·1**	**11·6**	**9·8**	**2·2**	**1·5**	**(+0·3)**	**(+0·9)**
d	*A*.	July 20-27	25·9	23·4	21·3	24·2	27·8	30·3	31·8	30·4
	B.	Aug. 3-7	17·2	15·3	15·7	17·6	17·7	19·2	30·5	29·9
	(Two bores only. Figures too high.)	Loss	**8·7**	**8·1**	**5·6**	**6·6**	**10·1**	**11·1**	**1·3**	**0·5**
e	*A*.	Aug. 14-21	27·7	23·6	31·7	19·8	21·2	26·3	30·6	30·7
	B.	Aug. 31—Sept. 7	21·8	20·5	23·6	32·1	27·3	30·7	31·6	31·5
		Loss	**5·9**	**3·1**	**8·1**	(*+12·3*)	(*+6·1*)	(*+4·4*)	(*+1·0*)	(*+0·8*)

NOTE.—Figures represent percentage of water as in Table I, but each averaged from three consecutive dates as there given.

The means of the three borings made immediately after irrigation have been taken together, in order to eliminate slight soil diversities; then, missing one boring, the means of the three preceding the next irrigation have been taken. The difference gives the loss of water from each layer during the number of days elapsing from group-centre to group-centre. This is then assumed to be a fair mean for the whole period between one irrigation and the next; in all probability the assumption is too low, since the stomata immediately after irrigation remain open longer each day, and so transpire more water, though this again may be partly compensated by the higher humidity of the air.

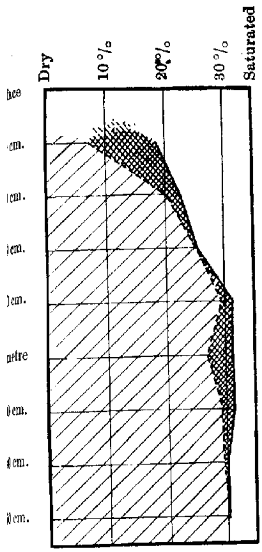
In any case the figure which we thus obtain for the mean water-loss from soil and plant between one irrigation and the next, is not likely to be too high.

In the case of the five periods here computed, this figure, expressed as a mean of the eight samples, and for an average day, worked out at 0·170, 0·359, 0·366, 0·541 and (+ 0·052) per cent. of the dry-weight of the soil. With the Sp. Gr. of the soil assumed to be 1·0, these figures correspond to *11, 24, 25, 36 and (+ 4) tons of water per acre per day.*

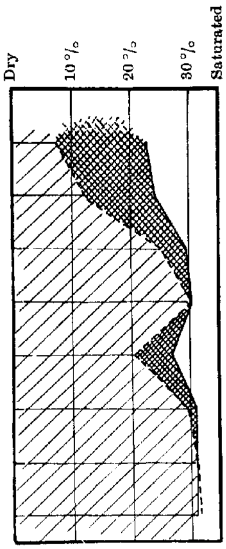
It remains to allocate this loss to soil-evaporation and to plant-transpiration respectively. For this there is no direct method, but fairly safe indirect conclusions may be drawn from the following considerations. At the beginning of this series of observations the plants are only a few centimetres in height, and most of the surface soil is freely exposed to the sun; the writer has published thermo-electric records[1] of the soil temperature near the surface under such conditions, at an earlier period of the season, showing how stringent the conditions are, and since the mean depth of the root-system at this time is only about 50—60 cm., it follows that a great part of the surface evaporation must be simply from the soil. Within the first two months—May and June—there is a very rapid growth of the stem, and a steady increase in root depth, so that by June 29th the root reaches a depth of about one-and-a-half metres, while the stem is over a metre in height. On this date it was noted that the surface soil of the plot was coated with Cyanophyeae (blue-green algae) which only flourish on surfaces where the evaporation is very low, or is continually compensated by fresh water supply. It would appear that the evaporation from the surface soil after July 1st was almost negligible, and it might be added that routine records taken with the hygrograph in this plot, at 10 cm. from the ground surface, showed that the mean humidity rose very rapidly during June and July, this implying a diminished evaporation, even without the reduction in wind velocity which follows from the interlacing of the plants.

Returning to the conditions during May, we see from the curves (Fig. 2) that even under these, the most stringent conditions, the loss of water is trivial below 40 cm. Making the maximum allowance for surface evaporation from the soil, in the light of these data it would

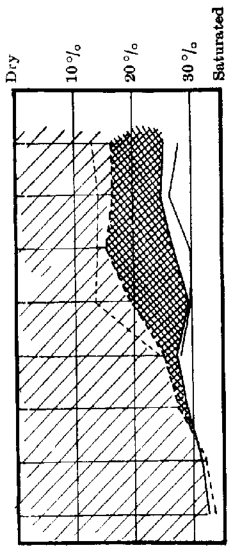
[1] *Loc. cit.*



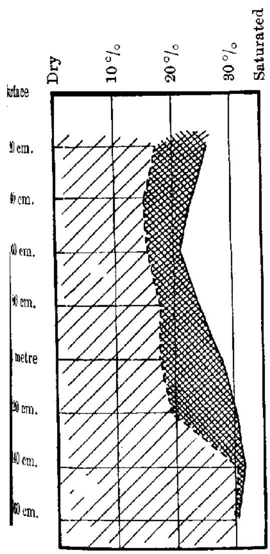
(*a*) May 8th—25th.
Stem, 10 cm. Root 50 cm.



(*b*) June 5th—19th.
Stem, 45 cm. Root, 90 cm.



(*c*) June 29th—July 13th.
Stem, 85 cm. Root, 130 cm.
To R. and L.—Single bores of June 26th and of July 17th.



(*d*) July 24th—Aug. 7th.
Stem, 120 cm. Root, 180 cm.

Fig. 2. Loss of water from soil between irrigations.

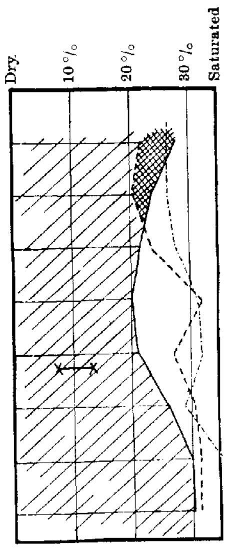
Plotted from Table II.

Each rectangle represents—

Vertically, the depth of soil.
Horizontally, the water-content at each depth.

Deeply-shaded portion represents the loss in each period, and is indirectly a crude picture of the root-system.

Mean height of stem and approximate depth of tap-root are indicated below each rectangle.



(*e*) Aug. 17th—Sept. 4th.
Stem, 130 cm. Root, 200 cm.
— · — · — On Sept. 25th.
x—x Water-table in bore on Sept. 26th.

seem more than fair to estimate that the correction should be somewhat as follows:

TABLE III.

Period	Total loss of water in tons per acre per day	Loss due to	
		Soil evaporation	Transpiration
May 8 to May 25 ...	11	7	4
June 5 to June 19 ...	24	10	14
June 29 to July 13 ...	25	4	21
July 24 to Aug. 7 ...	36	2	34
Aug. 17 to Sept. 4 ...	(+4)	?	

Mean transpiration = 18 tons of water per acre per day.

The figures, approximate though they may be, require some comment, in that M. Audebeau, who conducted the only extant series of experiments upon the transpiration of cotton, by weighing plants growing in large tanks, arrived at a mean figure for the same period of 21·9 tons per acre per day[1]. I have elsewhere pointed out[2] several serious objections attaching to this figure, notably that the plants were abnormally large and freely exposed to wind; yet, under normal and undisturbed field conditions, we have reached a figure of the same order as M. Audebeau's. It is one of the most curious examples of compensation of error with which I have yet met, even when allowance is made for the higher evaporimeter reading at Giza as against Korashia. Probably a detailed consideration of stomatal behaviour, of the wind velocity needed to accelerate transpiration, and so forth, would explain the convergence, but it is of more importance to notice that M. Audebeau's figure, now confirmed by the writer, was so high

[1] *State Domains Report*, Cairo, 1910. [2] *Loc. cit.*

that it necessitated more water for the cotton crop alone than was available in the Nile during the summer. We can only conclude that the deep-rooted plant draws on the water-table, when within reach.

We have now reached this last conclusion by three different lines, and it seems to be quite sound. In one way it is quite to be expected, but it does not seem to have been taken into account before, presumably because the "deep draught" of the plant had not been realised. The drying effect which is shown in this series at 120 cm. might easily, with a deeper water-table, have been followed down to two-and-a-half metres.

It does not follow that the water-table, though utilisable, is of any advantage to the crop above. The existence of a water-table within 3 metres of the surface still implies a limitation of the root-system, and a risk of submergence, with all their attendant evils.

GRADIENT OF HUMIDITY.

With the previous discussion of the "surface climate" conditions in mind, the reversal of humidity gradient shown in Fig. 2 becomes easily comprehensible.

The metaphor which the writer formerly[1] devised, of a "functional centre of gravity" in the root-system, becomes very convincing. It had not been suspected previously—to the best of the writer's belief—that the soil would actually become drier as the borer passed deeper into it, down to at least a metre depth.

The abnormal reversal of the gradient between August 14th and September 7th is also worthy of note. It is due solely to the rise of the water-table, but it is shown far above the water-table, and many days before the saturation level actually reached the samples. We might assume that this upward movement of the water was due to capillarity, but it is quite debatable, and indeed highly probable, that the movement is rather a case of direct "thrust" of the rising water upon the overlying water-films, producing a general redistribution of hydraulic pressure.

Had it not been for the patches of clay on the site examined, it might be possible to make some physical calculations from the data at hand.

[1] *Loc. cit.*

EFFECT OF IRRIGATION.

Consecutive with the last paragraph it may be well to comment on the effect of applying surface water, by irrigation, as shown in these records.

I am informed by local chemists and physicists that some divergence of opinion exists as to the depth at which the effect of surface irrigation is noticeable in the soil. The general conclusion appears to be that the effect is only felt over a limited distance; this is the exact contrary of my own deduction from these records (Fig. 2), and is, to say the least, extremely improbable. The writer is not sufficiently qualified to discuss the matter physically, but it might be pointed out that the introduction of a new component—irrigation water—at the upper end of a system which is in equilibrium between surface-tension, transpiration and gravity, can hardly fail to produce a disturbance throughout the system. Any contrary conclusion must be due to some local abnormality, or to methods which are not sufficiently minute to detect the disturbance.

Inspection of the curves in Fig. 2 shows at once that the effect of each irrigation is felt to the lowest level at which the soil has dried, and that the level at which its effect disappears is that at which no change has been taking place in the soil water-content. Had the water-table been deeper, the effect would undoubtedly have been traceable to a greater depth. But it should be noted that this effect is often of short duration, owing to the rapid desiccation of the soil brought about by the roots, and unless the observations are, as in this case, made at frequent intervals, is likely to escape detection altogether.

THE PROBABLE ERROR OF SOIL-WATER DETERMINATIONS.

In connection with the previous heading it is of interest to note that very serious errors may arise in the determination of the water condition (and indirectly, of the salt-content) of fields of cotton in Egypt by any method of sampling, if the sampling is intermittent.

The present data are scarcely full enough to enable a statistical statement to be made, but the following result will illustrate the writer's meaning.

The water-content of the samples at 40 and at 60 cm. taken at 13 and at 17 days after watering, may be considered as equivalent

to a series of samples taken half a metre down, over a large field at fifteen days after irrigation, for the purpose of ascertaining its water condition, since irregularities of soil surface and sub-soil structure, which are practically absent in the small plot observed by the writer, would then be involved. If no allowance is made for the seasonal change, shown in this present paper, the P.E. of these samples works out to 3·1 per cent. of actual soil-water.

Similarly, the samples from 100 and 120 cm. give a P.E. of 3·5 per cent. of actual soil-water.

The extreme variation in soil water-content, excluding the water-table, ranges in these experiments from 33 to 10 per cent. At the usual odds of 30 : 1 for certainty ($3{\cdot}5 \times 3{\cdot}2 = 11\frac{1}{2}\,^{\circ}/_{\circ}$), the samples at one metre depth may range from one extreme to the other without being significant.

It is highly improbable that any conclusions would ever be drawn from single samples taken blindly in this way, but it is well to bear in mind that the seasonal change should be the first consideration, the post-irrigation change the second, and that totally false estimates might be formed by such random sampling.

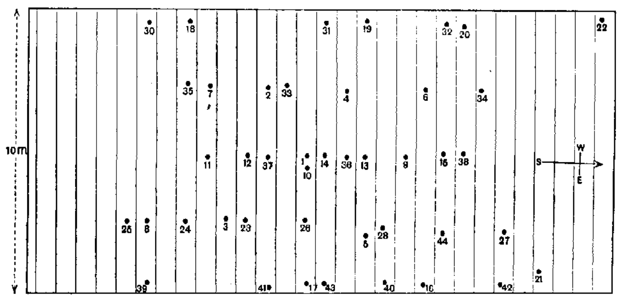


Fig. 3. Plan of Bores. Lines E. and W. represent ridges.

CONCLUSION.

This paper describes and discusses a series of soil-water determinations made on an area of 20 × 10 metres in a field of cotton at Giza, in Egypt.

The especial feature of the determinations was their frequency. They were made every three and four days alternately, and at twenty centimetre intervals down to 160 cm.

Fairly smooth statistical results were thus obtained.

The chief conclusions drawn are as follows:

A. The depth of the root may be roughly traced by its drying effect on the soil.

B. This drying, combined with a change in the surface climate, causes a reversal of the Humidity Gradient, so that deep soil is drier than surface soil in September.

C. The rate of evaporation from this field of cotton plants averaged about 20 tons of water a day, from May to October.

The results of M. Audebeau in this respect are substantially correct, though circumstantially in error.

D. Application of irrigation water to the surface is felt to an indefinite depth. Absence of evidence to this effect is due to imperfection of method.

E. Determination of soil water-content in an Egyptian cotton field by random sampling is almost worthless, unless due regard is paid to the seasonal variation.

F. The water of the water-table, when within two metres of the surface, may be utilised by the crop, but this conclusion does not vitiate any previous conclusions drawn by the writer as to the prejudicial effects of saturated soil.

G. A rise of the water-table is analogous to surface irrigation, and there is some indication of a direct hydraulic thrust in both cases.

The observations and operations were entirely conducted personally until July 18th, when they were taken over by Mr F. S. Holton during my absence on leave. I am very much indebted to Mr Holton for contriving to carry on the extra work in a very busy summer.

THE EFFECT OF BASTARD TRENCHING ON THE SOIL AND ON PLANT GROWTH.

By SPENCER UMFREVILLE PICKERING, M.A., F.R.S. and E. J. RUSSELL, D.Sc. (Lond.).

(*Rothamsted Experimental Station.*)

Bastard Trenching as ordinarily performed consists of two distinct operations; loosening the lower spit of soil, and digging into it farmyard manure or other fertilising material. A considerable volume of data has been accumulated to show the effect of the addition of farmyard manure to soil, but little is known of the effect of loosening the bottom spit, either on the soil or on the plant.

The experiments described in this paper were made on plots that had been bastard trenched to a depth of three spits, but not manured. The first and second spits were put back in their natural order, but no manure was added. The experiment, therefore, deals simply with deep cultivation effect, and is not complicated by any disturbing factors due to the operation of the manure.

Four distinct types of soil were investigated; a light sand, two loams (both rather heavy) and a strong clay. The bulk of the experiments extended over the four seasons from March 1909 to the end of 1912, a period which included the unusually hot dry summer of 1911, the cold wet summers of 1909 and 1912, and the season 1910 which was of intermediate character.

Samples of soil were periodically taken for determinations of moisture and nitrate, and observations were made on the growth of fruit trees in the plots.

The results show that trenching has very little effect on the moisture content of the soils. There is some indication that it facilitates percolation from the surface to the subsoil on heavy loams and clay, but the indication is not very marked, and only comes out with certainty

on looking over the whole of the results. It also somewhat increases the subsoil moisture. No other tendency could be distinguished, and we must regard trenching as a very minor factor in determining the water supply to the plant.

Nor did it appear to lead to any marked increases in the store of nitrate in the soil. There were small gains on the sand and rather larger gains on the clay, which persisted over all the four seasons, but these were never very great, and not much above the error of the experiment. Trenching did not appear to alter the distribution of the nitrate between the surface and the subsoil.

The behaviour of the plant furnishes a sensitive index to the changes in the soil. Here, however, the indications are not much more definite than those given by the determinations of water and nitrate. An increased leaf-size (generally of small dimensions) is shown in three out of the four cases in which this feature was measured, but in the fourth there is a reduction; an increased growth was recorded in three cases, but a reduction in two cases; whilst in the two instances where there were records of crops, both are in favour of leaving the ground untrenched.

Putting together the whole of the evidence, the effect of bastard trenching on the soil *when unaccompanied by manuring* is seen to be only small. Beyond a tendency to facilitate the drainage of water from the top spit to the lower spit in the clays and the heavy loams, and slightly to increase the nitrates, no definite change seemed to be produced. The effect on the growth of trees appeared to depend largely on the character of the seasons following the trenching and planting, as was exemplified by the different results obtained in the same plot of ground after trenching in 1895, and after retrenching in 1910. The practical conclusion may be drawn that bastard trenching by itself, done without addition of manure to the bottom spit, is not likely to bring about any sufficient change in the soil to justify the trouble and expense of the operation. Of course if there is a pan to be broken the case is different; but, where there is no pan, the main use of bastard trenching seems to be that it affords an opportunity for adding manure or other fertilising material to the bottom spit. Unless advantage is taken of this, the real benefit of the process is missed.

Experimental.

In all the present experiments the first and second spits of soil were removed, the third spit was broken up, but not removed, and then the second and first spits were replaced in their natural order. Nothing was buried in the trench.

The trenched and untrenched plots were contiguous to each other, and were similarly treated as to cultivation and manuring, a general artificial manure being applied in all cases, except in the experiment at Rothamsted.

The Soils.

(*a*) *The Sand.* This was situated at Millbrook, Bedfordshire, on the greensand formation, where the soil is very poor and sandy. The upper and lower spits differ very little in mechanical composition or in texture. There were three trenched plots here, with the corresponding untrenched plots adjoining; one (first series) was trenched in February 1909, another (second series) in March 1910, and the third in March 1912. In the first two cases the plots measured 100 square feet, the third was 484 square feet. The second plot was added to ascertain whether the results obtained on the first plot had been affected by the lie of the ground; it was therefore arranged so that the two trenched and the two untrenched plots should lie diagonally towards one another, as indicated in the following diagram:

Trenched	Untrenched
Untrenched	Trenched

The mechanical analysis of the soils is given in Table I.

(*b*) *The Loams.* Two sets of experiments were carried out, one at the Woburn Experimental Fruit Farm, Ridgmont, the other at Rothamsted. The Ridgmont soil is a heavy loam situated on the Oxford clay. It was trenched in 1894 and retrenched in 1910. Each plot measured 250 ft. by 12 ft. The Rothamsted plots were situated

on a part of Broadbalk field where lucerne, preceded by potatoes and wheat, had been grown. The central plot, measuring 80 ft. by 20 ft., was trenched in February 1909, the untrenched plot, of the same total area, was subdivided so as to lie on either side of the trenched section. This soil is also heavy, and of the clay-with-flints formation overlying the chalk.

TABLE I. *Mechanical Analysis of Soils.*

	Sandy Soil (Millbrook)	Loam (Ridgmont)	Loam (Rothamsted)	Clay (Ridgmont)
		Surface		
	%	%	%	%
Fine Gravel	3·2	1·0	1·3	0·4
Coarse Sand	68·2	54·8	9·5	8·6
Fine Sand	9·2	11·5	22·7	3·3
Silt	5·6	11·8	32·3	9·1
Fine Silt	4·9	6·7	8·9	11·1
Clay	5·0	9·3	12·9	43·0
Loss on Ignition	4·2	5·3	7·7	18·6
		Subsoil		
Fine Gravel	2·2	1·6	2·6	0·2
Coarse Sand	70·8	53·9	8·3	16·8
Fine Sand	8·8	11·5	24·5	10·7
Silt	4·9	10·9	27·5	10·8
Fine Silt	5·0	4·7	10·6	10·0
Clay	5·2	10·9	15·0	32·0
Loss on Ignition	4·2	5·2	7·1	13·8

(*c*) *The Clay.* This is a heavy clay soil, on the Oxford clay formation at Ridgmont, and shows the unusual phenomenon of containing more fine material in the surface than in the subsoil. As it is very heavy and low-lying, it was drawn out into ridges and furrows, the former, on which the trees were planted, being 7 ft. 6 in. apart. This experiment really deals with the effect of retrenching, because the ground had all been trenched in 1894; the portion which was retrenched in November 1905 lay on the side nearer to a brook.

The Trees.

(*a*) *In the sandy soil.* In the three trenched plots on the light Millbrook soil, dwarf trees, of five different varieties, two years of age, were planted in 1912: five in each of the earlier trenched plots, and 40 in the last trenched one. A like number of trees were planted in

the corresponding untrenched plots. Details as to the behaviour of these trees in the following season are entered in Table V, the results with the different varieties being given separately in the case of the larger plots only.

(*b*) *In the loams.* The whole of the ground at the Fruit Farm, Ridgmont, except one plot measuring 250 by 50 feet, was trenched in the summer of 1894. In the untrenched plot 18 dwarf apple trees of three different varieties—Bramley, Cox and Potts—were planted in the following winter, and the general behaviour of similar trees in four other plots in the adjoining trenched ground, as compared with that of the trees in the untrenched plot, is illustrated by the data in Table V (Ridgmont, first series). In 1910, after these trees had been removed, 40 two-year-old trees of Gascoyne's Scarlet Seedling were planted in the portion which had never been trenched, and 40 in the adjoining plots trenched in 1894, but one half of these plots were retrenched in 1910, immediately before the planting. This constituted the second series. The trees in this case were rammed on planting, but they were not so in any of the other experiments. Though this ramming might naturally be expected to have diminished any effect due to the trenching, it will be seen that this, apparently, has not been the case, these trenched plots showing a greater effect due to the trenching than in any other instance.

At Rothamsted the plots were planted in February 1909 with eight standard and 30 dwarf apple trees, six dwarf pears and six dwarf plum trees, 20 gooseberry and 20 currant bushes. No manure was supplied.

(*c*) *In the clays.* Four standard and five dwarf apple trees, four standard and five dwarf plum trees, and five dwarf pear trees were planted in December 1905.

The Moisture Determinations. Samples were taken in the field by the cylindrical borer and allowed to dry in a hot chamber at 37°—40° C. The mean results for the various soils over the whole period are given in Table II, and the fuller results in Table III. In the latter table no significance is attached to differences less than $\pm$ 0·3 per cent. in the case of the sands and loams, or $\pm$ 0·6 per cent. in the case of the clay, experience having shown that variations of this order come within the limits of experimental error. The small effect of trenching on the moisture content is evident. The trenched and untrenched plots on the sand are practically equally moist, when the results of the two sets of plots are considered. In Series I the trenched plot has

a drier subsoil than the untrenched, but not in Series II. On the loam the trenched surface soils tend to be distinctly moister in dry weather (*e.g.* August 1911 and May 1912) and are on some occasions rather drier in wet weather (*e.g.* September and November 1909, January 1912); the trenched subsoil in Series II is, on the other hand, somewhat wetter in wet weather (indicating increased percolation) and drier in dry weather than the untrenched. At Rothamsted the trenched soil is throughout the moister. On the clay soil the consistent behaviour is seen in the subsoil, which is always wetter on the trenched plots than on the untrenched. This result may be partly due to the relative positions of the plots, the trenched plot, as already stated, lying towards the stream. But going over the whole of the figures there is an unmistakable though small tendency for trenching to increase the subsoil moisture. The surface soil is sometimes wetter on the trenched plot, but in dry weather it is often markedly drier.

TABLE II. *Mean Percentages of Moisture in the Trenched and Untrenched Soils.*

	1909		1910		1911		1912		Mean	
	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched
Sandy Soil										
Millbrook, 1st Series. Top 9″	11·9	11·9	8·4	8·4	4·8	4·1	6·7	6·0	7·9	7·5
,, ,, 9″–18″	8·8	9·9	7·4	7·5	4·5	4·6	5·9	7·6	6·5	7·4
,, 2nd Series. Top 9″	—	—	9·0	8·8	4·6	4·8	6·1	6·8	6·1	6·4
,, ,, 9″–18″	—	—	6·6	6·2	4·2	4·1	5·6	5·2	5·2	5·0
Loam										
Ridgmont, 1st Series. Top 9″	14·0	14·1	12·2	11·6	7·5	7·2	9·6	9·7	10·5	10·5
,, ,, 9″–18″	13·3	13·2	11·7	11·2	8·0	7·9	9·5	10·1	10·9	10·5
,, 2nd Series. Top 9″	14·2	14·2	11·6	11·2	8·7	8·1	10·2	9·2	10·8	10·2
,, ,, 9″–18″	14·7	14·3	13·1	12·6	9·7	10·5	11·3	10·7	11·8	11·7
Rothamsted. Top 9″	15·3	15·1	14·9	14·2	12·8	12·1	13·7	13·6	14·1	13·5
,, 9″–18″	16·2	15·3	15·4	14·6	13·9	12·8	15·1	14·0	15·1	13·9
Clay										
Ridgmont Ridges. Top 13″	28·2	27·7	23·4	23·6	16·8	18·7	18·2	19·6	21·4	22·4
,, ,, 13″–22″	32·1	29·2	28·4	27·5	23·8	20·5	23·1	22·2	26·6	24·8
,, Furrows. Top 5″	28·0	27·1	21·9	23·6	19·3	20·3	21·1	20·3	22·7	22·7
,, ,, 5″–14″	33·1	31·4	30·6	28·9	25·2	22·6	25·0	23·8	28·2	26·3

TABLE III. *Water present at different dates in the Trenched and Untrenched Soils.*
Percentage of Water.

Description	Date of Trenching	1909 March 13th	1909 May 6th	1909 July 14th	1909 Sept. 1st	1909 Sept. 17th	1909 Nov. 17th	1910 June 14th	1910 Aug. 2nd	1910 Sept. 11th
Sandy Soil										
Millbrook. 1st Series. Top 9″ Untrenched	Feb. 1909	14·0	9·4	12·1	9·4	12·4	14·2	8·4	9·7	7·2
,, ,, ,, Trenched		14·0	10·1	11·4	9·1	12·8	14·2	8·7	9·0	7·6
,, ,, 9″—18″ Untrenched		10·5	9·1	11·6	8·8	7·6	11·9	7·4	8·4	6·8
,, ,, ,, Trenched		10·0	8·0	9·6	6·8	7·8	10·4	7·5	7·9	6·9
,, 2nd Series. Top 9″ Untrenched	Mar. 1910	—	—	—	—	—	—	9·0	8·9	8·4
,, ,, ,, Trenched		—	—	—	—	—	—	9·1	9·4	8·6
,, ,, 9″—18″ Untrenched		—	—	—	—	—	—	6·3	6·6	5·8
,, ,, ,, Trenched		—	—	—	—	—	—	6·1	7·1	6·5
Loam										
Ridgmont. 1st Series. Top 9″ Untrenched	1894	14·6	11·2	13·7	14·0	15·0	16·2	11·6	11·9	11·4
,, ,, ,, Trenched		14·4	—	13·4	13·4	14·2	14·7	11·3	12·7	12·1
,, ,, 9″—18″ Untrenched		13·9	12·8	13·0	12·6	12·9	14·0	12·0	11·3	10·3
,, ,, ,, Trenched		13·9	—	13·6	12·2	13·0	13·7	11·2	12·1	11·7
,, 2nd Series. Top 9″ Untrenched	Retrenched 1910	—	—	13·2	13·0	14·4	16·3	11·0	11·4	11·3
,, ,, ,, Trenched		—	—	12·9	13·0	14·7	16·0	11·6	11·8	11·6
,, ,, 9″—18″ Untrenched		—	—	12·6	15·8	13·2	15·5	11·8	13·2	12·9
,, ,, ,, Trenched		—	—	12·8	16·5	14·3	15·1	13·1	13·1	13·1
		April 6th	May 7th	July 6th	Oct. 28th			May 30th	July 27th	Sept. 20th
Rothamsted. Top 9″ Untrenched	1909	15·0	14·3	13·7	17·7	—	—	13·5	14·3	14·9
,, ,, Trenched		15·5	13·7	14·3	17·9	—	—	13·6	15·1	15·1
,, 9″—18″ Untrenched		15·4	14·3	14·7	17·3	—	—	14·7	13·9	15·2
,, ,, Trenched		15·6	16·0	15·0	18·2	—	—	15·0	15·4	15·7
Clay		March 13th	May 6th	July 14th	Sept. 1st	Sept. 17th	Nov. 17th	June 14th	Aug. 2nd	Sept. 11th
Ridgmont. Ridges. Top 13″ Trenched	1894	27·3	25·7	27·5	27·6	26·5	31·9	24·1	22·7	24·0
,, ,, ,, Retrenched	1905	29·5	24·7	27·7	28·4	26·9	31·3	19·7	24·6	25·8
,, ,, 13″—22″ Trenched	1894	30·7	26·7	28·3	29·1	27·3	33·3	27·2	27·5	27·7
,, ,, ,, Retrenched	1905	33·7	31·6	30·3	31·4	31·3	34·1	28·3	29·3	27·7

TABLE III—(*continued*).

Description	Date of Trenching	1911 May 4th	May 22nd	June 15th	July 13th	Aug. 9th	Sept. 7th	1912 Jan. 13th	April 1st	May 3rd	June 26th	July 19th	Sept. 27th
Sandy Soil													
Millbrook. 1st Series. Top 9″ Untrenched ...	Feb. 1909	8·4	7·2	2·8	1·5	1·3	3·3	9·5	9·4	4·4	5·6	2·2	4·9
,, ,, ,, Trenched		9·2	6·8	3·0	4·1	1·2	4·3	10·2	9·4	6·8	5·9	3·1	4·9
,, ,, 9″—18″ Untrenched ...		6·9	7·5	4·1	3·1	1·9	4·4	8·9	9·2	(6·6)	6·5	4·9	8·6
,, ,, ,, Trenched		6·6	7·2	3·9	5·7	3·0	5·0	7·8	7·6	4·2	6·6	6·9	6·2
,, 2nd Series. Top 9″ Untrenched ...	Mar. 1910	9·5	8·1	4·2	1·1	2·4	3·8	(8·0)	9·8	8·4	6·6	2·3	6·9
,, ,, ,, Trenched		9·4	8·2	3·5	1·7	1·3	3·5	9·1	9·2	4·6	5·5	2·5	5·9
,, ,, 9″—18″ Untrenched ...		6·1	6·1	—	1·7	2·6	4·2	7·0	7·7	4·0	5·3	1·8	5·2
,, ,, ,, Trenched		6·5	6·9	3·7	1·3	2·0	4·7	(7·9)	8·0	—	6·0	3·1	5·5
Loam													
Ridgmont. 1st Series. Top 9″ Untrenched ...	1894	11·1	9·9	8·7	6·1	3·4	4·4	16·3	10·8	4·2	10·5	6·5	9·7
,, ,, ,, Trenched		10·6	9·8	8·4	7·3	4·5	4·5	14·6	9·9	6·6	10·0	7·6	8·8
,, ,, 9″—18″ Untrenched ...		11·4	10·4	9·3	7·3	4·0	5·1	14·6	10·6	6·7	10·8	8·6	9·7
,, ,, ,, Trenched		11·1	10·5	8·7	8·1	4·8	5·0	14·5	10·7	6·1	9·0	7·2	(10·7)
,, 2nd Series. Top 9″ Untrenched ...	Retr. 1910	11·5	9·6	8·3	7·3	5·8	6·2	15·7	10·7	6·6	9·1	6·2	6·7
,, ,, ,, Trenched		11·2	9·6	9·5	7·6	7·3	7·2	16·8	10·4	8·4	9·7	6·6	9·1
,, ,, 9″—18″ Untrenched ...		11·7	12·8	11·4	10·5	7·4	9·3	15·1	10·1	9·1	10·3	9·9	9·8
,, ,, ,, Trenched		11·8	11·9	9·8	8·3	8·3	7·9	16·3	11·0	8·7	11·2	9·6	10·7
		May 17th	June 8th	July 27th	Sept. 13th			Feb. 15th	April 30th	May 30th	June 26th	July 22nd	Sept. 25th
Rothamsted. Top 9″ Untrenched	1909	15·6	12·8	13·0	6·8	—	—	18·2	11·0	10·7	14·0	14·6	13·4
,, ,, Trenched		16·1	14·0	13·7	7·6	—	—	17·9	11·1	11·7	14·3	15·3	12·3
,, 9″—18″ Untrenched		16·4	14·0	13·2	7·5	—	—	17·5	12·6	12·1	14·4	13·3	14·1
,, ,, Trenched		16·9	14·4	13·8	10·4	—	—	18·2	14·4	13·3	15·3	14·6	14·7
		May 4th	May 22nd	June 15th	July 13th	Aug. 9th	Sept. 7th	Jan. 13th	April 1st	May 3rd	June 26th	July 19th	Sept. 27th
Clay													
Ridgmont. Ridges. Top 13″ Trenched	1894	24·5	20·7	19·8	18·2	13·0	15·7	27·5	20 8	(16·2)	17·5	14·9	17·5
,, ,, ,, Retrenched	1905	23·3	15·3	18·2	16·6	14·4	12·8	28·5	19·0	15·1	18·4	13·9	14·1
,, ,, 13″—22″ Trenched	1894	23·8	24·5	24·6	16·0	16·6	17·3	29·7	25·4	(20·6)	21·1	17·7	17·2
,, ,, ,, Retrenched	1905	27·1	26·4	26·7	22·0	20·5	20·0	30·8	26·1	22·4	20·0	19·7	19·8

The Nitrate Determinations. A known weight of the dried soil (150—200 grams) was pounded up and extracted with about 800 c.c. of water on a Büchner funnel, the extract was concentrated in presence of a little magnesia, acidified and reduced by the zinc-copper couple. The ammonia was then determined by distillation into standard acid in the usual way. Table IV gives the average results for the different years. No significance attaches to less than one part per million in the case of sands and loams, or two parts in the case of clays. This is for

TABLE IV. *Mean parts per million of Nitrates in the Trenched and Untrenched Soils.*

	1909		1910		1911		1912		Mean	
	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched	Trenched	Untrenched
Sandy Soil										
Millbrook. 1st Series. Top 9″	5	4	4	2	5	4	4	3	4	3
,, ,, 9″—18″	4	3	3	2	3	4	3	2	3	3
,, 2nd Series. Top 9″	—	—	7	7	4	4	7	5	6	5
,, ,, 9″—18″	—	—	5	4	3	3	3	3	4	3
Loam										
Ridgmont. 1st Series. Top 9″	3	6	2	3	9	9	8	4	6	6
,, ,, 9″—18″	3	3	2	3	5	5	4	3	4	4
,, 2nd Series. Top 9″	5	5	10	11	15	14	4	6	8	9
,, ,, 9″—18″	4	3	6	4	10	9	3	4	6	5
Rothamsted. Top 9″	6	6	12	13	15	14	7	7	9	10
,, 9″—18″	6	6	7	7	12	13	6	6	8	8
Clay										
Ridgmont. Ridges. Top 13″	7	6	8	7	15	11	10	6	11	8
,, ,, 13″—22″	8	7	7	5	10	7	10	5	9	6
,, Furrows. Top 5″	5	5	7	6	10	6	6	3	7	5
,, ,, 5″—14″	4	4	7	3	8	5	5	3	6	4

the individual determinations: for the average results given in the table a difference of one part per million is probably real. The effect of trenching on nitrate production is only clearly marked in the clay soils; here it generally leads to an increase in the stock of nitrate both in the surface and the subsoil. It is difficult to account for the increase in the surface soil. The increase in the subsoil, when it occurs, may be associated with the increased aëration following after trenching; it is less likely to be due to the increased percolation. We could find but

little definite evidence that the small extra percolation on the trenched ground really affected the distribution of the nitrates.

The Amount of Plant Growth. The mode of taking the leaf size and the length of new wood formed is fully described in the *Report of the Woburn Experimental Fruit Farm* (1900, p. 114). Briefly, the leaf size is determined by weighing the sixth leaves from the ends of the shoots, while the length of new wood is determined by direct measurement. As the results show considerable variation in passing from tree to tree they are calculated as proportionate percentages, *i.e.* the values for the trenched and untrenched plots are calculated as percentages of the smaller result, whichever it happens to be, and then designated + or − according as the trenched plot gives the higher or the lower result. The increase in weight of the tree is first calculated as a percentage of the original weight and then worked out as percentages of the smaller result.

Owing to the variation in the crops from the individual trees or bushes it becomes necessary for the determination of these means to weight the individual results according to the actual magnitude of the crop. This has been done for the three values given in Table V.

TABLE V. *Effect of Trenching on the growth of Fruit Trees.*

A. *Sandy Soil.* Ground trenched 1909—1912 as stated. Trees planted 1912.

	Percentage difference in favour of trenching					
	Leaf size		Length of new wood		Increase in weight of the trees	
Trenched in 1909		+11		−54		−19
,, 1910		−4		−20		+41
,, 1912		—		—		—
Gascoyne	+2		−43		−33	
Grosvenor	−3		+31		−85	
Worcester	+11	+6	−43	−36	+35	−7
King	+35		−67		+33	
Newton	−14		−56		+13	
Mean		+4		−37		+5

B. *The Loams.* I. Ridgmont. 1st Series, 1894—1904 and 1908. Ground trenched during the summer of 1894. Trees planted winter 1894.

Feature examined	Percentage difference in favour of trenching
Leaf size : 10 years 1895—1904	+10
Size of the trees in 1899	+4
,, ,, 1904	+4 } + 3
,, ,, 1908	+2
Weight of the trees after 11 or 15 years ...	+ 3
,, ,, during first 10 years ...	−21

Average of height, spread of branches and girth of stem.
Results with Bramley and Cox alone available.

2nd Series. Same ground but part was retrenched in 1910 and replanted immediately afterwards.

	Percentage difference in favour of trenching						
	Leaf size			Length of new wood			
	1911	1912	Mean	1910	1911	1912	Mean
Trenched in 1894 ...	−16	−11	−14	+ 28	+15	+25	+23
Retrenched in 1910	− 3	+ 3	0	+100	+61	+56	+72

II. Rothamsted. Ground trenched Feb. 1909. Trees planted directly afterwards.

Trees	Percentage difference in favour of trenching									
	Length of new wood					Weight of crops				
	1909	1910	1911	1912	Aver.	1909	1910	1911	1912	Aver.
Standard Apples A. (4)	+ 48	− 37	− 14	− 18	− 5	—	+273	+108	− 3	+126
,, ,, B (4)	+ 7	+ 36	+ 1	+ 1	+ 11	—	+ 91	—	+77	+ 84
Dwarf Apples A. (10) ...	+ 48	+ 21	+ 5	− 4	+ 17	—	− 7	+ 80	−36	+ 12
,, ,, B. (10) ...	+ 7	− 34	− 20	− 40	− 22	—	− 24	+ 27	−32	− 9
,, ,, C. (10) ...	+ 24	+ 36	− 59	− 16	− 4	—	+ 19	+ 74	−30	+ 21
Dwarf Pears (6)	− 12	− 9	− 4	− 28	− 13	—	—	—	—	—
,, Plums (6)	+ 11	+ 29	+ 35	+ 85	+ 40	—	—	—	—	—
Gooseberries A. (10)	+ 36	−166	−148	− 6	− 71	+31	+ 12	+ 33	+30	+ 26
,, B. (10)	−113	−122	− 52	+ 27	− 65	− 6	− 6	− 29	−25	− 17
Black Currants A. (10) ...	− 64	−187	−128	−189	−142	(+51)	—	−103	−23	− 25
,, ,, B. (10) ...	− 9	− 78	− 56	−118	65		—	− 79	−78	− 35
Mean of large fruit	+ 18	+ 6	− 8	− 3	+ 3	—	—	+ 40	−28	+ 4
,, small ,, ...	− 50	−136	− 96	− 72	− 88	+14	− 1	− 38	−22	− 12
General mean	− 5	− 46	− 40	− 28	− 30	—	− 1	+ 1	−25	− 8

C. *The Clay.* Both plots trenched 1894, one retrenched Nov. 1905. Trees planted Dec. 1905.

	Percentage difference in favour of trenching — Leaf size							
	1906	1907	1908	1909	1910	1911	1912	Average
Apples, Standard	—	+16	+11	+ 16	− 4	+ 6	+6	+ 8
Plums ,,	—	+20	−20	+ 23	—*	—*	—*	+ 8
Apples, Dwarf	—	+27	− 4	+ 75	+38	+ 7	+9	+25
Pears ,,	—	+ 2	+ 4	− 16	− 8	+23	+6	+ 2
Plums ,,	—	+ 1	−11	−138	− 3	+30	0	−20
Mean	—	+13	− 4	− 8	+ 6	+16	+5	+ 5

	Length of new wood							
	1906	1907	1908	1909	1910	1911	1912	Average
Apples, Standard	+47	+38	+26	—	+52	+12	− 92	+14
Plums ,,	+49	+64	+71	—	—*	—*	—*	+61
Apples, Dwarf	+23	+21	+ 4	—	+46	−19	−138	−11
Pears ,,	+13	+25	+92	—	+ 8	+37	− 67	+23
Plums ,,	+15	+13	+28	—	+19	−32	−122	−13
Mean	+35	+32	+44	—	+31	− 1	−105	+15

* Plums omitted as those in one of the plots had died.

The results are all summarised in Table VI. Speaking generally, the values for tree growth and for the soil factors are fairly clearly connected. In the sandy soil (first series), where water supply is apt to be a limiting factor, we find a drier subsoil and a reduced tree growth, though the results of the second series make it more than doubtful whether the drier subsoil was really due to the trenching. In the loam (first series) at Ridgmont, trenching has had no persistent effect on the soil except somewhat to increase the moisture content of the subsoil, it has also had only a small favourable effect on the tree. On the clay, where the nitrogen supply assumes considerable importance, there is a small gain in nitrate on the trenched plot, and a small gain in growth. There are, however, two sets of results where no connection can be traced; the trees in the Ridgmont loam (second series) and the bush fruits at Rothamsted. At Ridgmont (second series) there is a large proportionate gain in tree growth on the trenched plot which seems too much to attribute to the small gain in moisture content, while at

TABLE VI. *Summary of results showing effects of Trenching.*

	Effect on Soil		Effect on plant growth		
	Gain in percentage of water present	Gain in nitrate parts per million	Gain in leaf size; proportionate percentage	Gain in growth*; proportionate percentage	Gain in crops; proportionate percentage
Sandy Soil					
1st Series	Surface +0·4	+1	+4	−16	−21
,, ...	Subsoil −0·9	Nil			
2nd Series	Surface −0·3	+1	—	—	—
,, ...	Subsoil +0·2	+1			
Loam					
Ridgmont					
1st Series	Surface Nil	Nil	+10	+ 3	—
,, ...	Subsoil +0·4	Nil			
2nd Series	Surface +0·6	−1	—	+72	—
,, ...	Subsoil +0·1	+1			
Rothamsted					
	Surface +0·6	−1	—	+ 3‡ −88§ } −30‖	−8‖
	Subsoil +1·2	Nil	—		
Clay †					
	Surface −1·0	+3	+5	+15	—
	Subsoil +1·8	+3	—	—	—

The sign + is used where the result is in favour of trenching and − where it is against trenching.

* Means of length of new wood and increase in weight of the trees.

† Retrenched ground compared with ground trenched 12 years previously.

‡ Large fruits only. § Small fruits only.

‖ Large and small fruits together.

Rothamsted there is an even greater effect produced on bush fruit, but in the other direction, which, again, would not have been anticipated from the small moisture gain. The trees at Ridgmont had been rammed into the soil on planting but this treatment had been applied equally on the trenched and the untrenched ground; while the bush fruit at Rothamsted had been grown without farmyard manure (known to be very desirable for these plants) and therefore had made rather poor growth; again, however, the same treatment had been applied both to the trenched and untrenched ground. Apart from these two cases (which we do not propose to discuss in detail) trenching is seen

to have no greater effect on the development of the tree than it has on the moisture and nitrate content of the soil; indeed, if a general mean is taken of all the plant data, it is found to equal -1 per cent. in favour of trenching.

However, the details of the experiments indicate that there were differences between the trees on the trenched and the untrenched plots, the trenching sometimes acting favourably and sometimes unfavourably. The character of the season immediately following the trenching and the planting seems to play an important part; thus on the Ridgmont clay (Table V, c) the results obtained during the first three years were strongly in favour of trenching, whilst those of 1912 were still more strongly against it. Again, on the Ridgmont loam the trenching done in 1894 was practically without effect on trees planted immediately afterwards, while that done in 1910 had a very marked effect on the trees then planted, which, however, belonged to a different variety. These effects are difficult to explain, and are not uniform in one direction; indeed, to a considerable extent they eliminate themselves when a mean is taken. Hence they do not affect our general conclusion that trenching, when unaccompanied by manuring of the bottom spit, has only a small effect, either on the soil moisture, the soil nitrates, or the growth of the tree.

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